Local anesthetics: New insights into risks and benefits
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Citation for published version (APA):

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Local anesthetics
New insights into risks and benefits

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Local anesthetics

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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom

ten overstaan van een door het college voor promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op woensdag 11 juni 2014, te 14:00 uur

door Philipp Lirk
geboren te Salzburg, Oostenrijk
Promotiecommissie

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Section 1

General Introduction and Thesis Outline
Chapter 1.1

Outline of Thesis
A brief history of Regional Anesthesia

Local anesthetics (LA) are indispensable in contemporary regional anesthesia and pain management. Introduced in Western medicine in the late 19th century, the prototype substance cocaine had been widely used by South American cultures for thousands of years. However, these tribes were used to chewing coca leaves, which dried out during shipping to Europe, such that the real breakthrough of cocaine was made possible only by the isolation of cocaine in 1859. Cocaine was used in oral form for various psychiatric diseases and fatigue by, among others, Sigmund Freud. However, it was his colleague, Carl Koller, who is widely credited for the first experimental topical application of cocaine. Koller was ophthalmologist at the Vienna General Hospital, and had been looking for ways to alleviate the pain of cataract surgery.

The discovery of the topical numbing properties of cocaine led to the development of regional anesthesia in its modern form, with the basic principle that injection of cocaine next to nerves produces a transient and reversible interruption of pain propagation, sensory and motor function. Over the following decades, many types of nerve block and neuraxial (epidural and spinal) anesthesia and analgesia were introduced. However, insufficient materials and medications with a narrow therapeutic range hampered widespread acceptance. For example, the first published administration of continuous epidural anesthesia in 1949 was achieved by an urethral catheter introduced into the epidural space. Another anecdote is the first performance, in 1889, of spinal anesthesia by Bier, which was successful from a pharmacological point of view, but the large needle used led to days of severe post-dural-puncture headache.

The introduction of the first short-acting amide-type local anesthetic, lidocaine, in 1947, and the standard long-acting amide-type local anesthetic, bupivacaine, were pharmacological milestones in regional anesthesia. In parallel, improved needle and catheter equipment meant that regional anesthesia could be applied in a more reliable and safe manner. In the 1990s, ultrasound-guided regional anesthesia was coined by a group of physicians in the in the Vienna General Hospital, the very workplace where hundred years earlier Carl Koller had made his
ground-breaking discovery. Stephan Kapral and Peter Marhofer described the
guidance of the regional anesthesia needle by ultrasound, sparking renewed interest
in regional anesthesia techniques.  

If scientific publications can be taken as surrogate markers of clinical
relevance, regional anesthesia is experiencing a surge in technical advances. At the
same time, these publications reflect the quest to define evidence-based indications
for these blocks on a procedure- and patient-based foundation.

Contemporary focus

Regional anesthesia has become an integral component of the
anesthesiologist’s armamentarium. Perceived benefits include the reduction in
postoperative opioid demand, and attenuation of postoperative symptoms and
complications such as nausea and vomiting, and ileus. The endocrine stress
response to surgery can be substantially alleviated when regional anesthetic
techniques are employed. However, it is increasingly acknowledged that these
beneficial effects are not relevant in all types of surgery and patients. Many of the
beneficial effects of regional anaesthesia can be copied by the systemic
administration of local anesthetics. Current consensus is to consider epidural
anesthesia and analgesia in high risk patients, thoracotomy, and major upper
abdominal and vascular surgery, to treat most cases of lower abdominal and
laparoscopic surgery using multimodal analgesia, and to use peripheral regional
anesthesia for extremity surgery as targeted and distal as possible. Currently, a
large share of the current research efforts in Regional anesthesia is dedicated to
defining patient- and procedure-based indications for the different techniques.

Despite being accepted as a relatively safe anesthetic technique, toxic
effects of local anesthetics themselves can present a considerable risk. Importantly,
in a dose-dependent manner, every local anesthetic is potentially toxic to virtually
any kind of tissue. The question whether specific local anesthetics are more toxic
than others remains unanswered. For example, there are studies suggesting that
lidocaine is more toxic than bupivacaine in vivo while other studies found no
difference and clinical evidence suggests that lidocaine is involved in nerve damage more often than bupivacaine.\textsuperscript{12}

Epidemiological studies suggest that after neuraxial (spinal, epidural) anesthesia, neurological complications such as transient radicular irritation (back pain with radiation down one or both buttocks or legs occurring within 24 h after surgery\textsuperscript{13}) may follow up to 30% of spinal anesthetics\textsuperscript{13,14}, and devastating complications such as cauda equina syndrome (severe low back pain, lower extremity motor weakness and sensory loss, bladder dysfunction, bowel incontinence) affect roughly 1:8000 patients.\textsuperscript{12,15} This is in accordance with a recent retrospective analysis of more than 100,000 neuraxial anesthetics, in which the incidence of presumed neurological complication was 1:1000, but when imaging techniques were used to validate these assumptions, the incidence was 0.07:1000 (with a 95% confidence interval of 0.02 – 0.13/1000).\textsuperscript{16}

This may be of special importance in the collective of patients presenting with diabetic neuropathy. Patients in the latter collective frequently feature relevant cardiovascular or renal comorbidities that would predispose them to undergo many types of surgery under regional anesthesia. On the other hand, limited epidemiological and experimental evidence suggests that neuropathic nerves are more susceptible to local anesthetic-induced neurotoxicity.\textsuperscript{17,18}

In the past, our group demonstrated involvement of the p38 Mitogen Activated Protein Kinase (MAPK) (= MAPK 14) in local anesthetic-induced neurotoxicity in vitro and in vivo.\textsuperscript{11,19-23} In particular, the p38 is specifically activated in cell cultures incubated with lidocaine.\textsuperscript{23} In the Habilitationsschrift (Innsbruck Medical University, 2010), we proposed a General Hypothesis that exposure of primary sensory neurons to local anesthetics triggers opening of calcium channels, resulting in an increase in cytosolic calcium, leading to a subsequent activation of the p38 MAPK. Potential downstream modulators include the lipoxygenase family of enzymes. Furthermore, we postulated that when local anesthetic is deposited at the axon, these events unfold at the neuron’s periphery, without necessarily involving the cell body. We believe this causes not only an
immediate neuronal injury apparent within hours, but also a prolonged inflammatory response combined with sustained tissue damage still detectable after several days.²²

**Aims of this thesis**

On the basis of these previous results, the investigations forming the basis of the current thesis aimed to

- investigate and review the mechanism and management of side-effects or failures of neuraxial anesthesia;
- investigate nerve injury related to the performance of regional anesthesia, with special focus on patients with pre-existing neuropathy;
- investigate potential novel actions of local anesthetics on the epigenetic signature of tumour cells; and
- conclude with a weighing of risks and benefits of regional anesthesia using local anesthetics.

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Local anesthetics: an introduction

Based on
Lirk P, Picardi S, Hollmann MW
Local anaesthetics: the essentials
Eur J Anaesthesiol 2014 (invited review, in press)
This introductory section is based on an invited Review for the European Journal of Anaesthesiology, and seeks to address 10 essential questions regarding the clinical use of local anaesthetics. Local anaesthetics are a heterogeneous group of compounds which block voltage-gated sodium channels. Each drug is characterized by distinctive physicochemical properties. Sodium channel block is caused by conformational change and the creation of a positive charge in the channel lumen. Different local anaesthetics can reach the LA binding site from the cytoplasmic compartment (“classic hydrophilic pathway”), directly via the lipid membrane (“hydrophobic pathway”), or can enter via large-pore channels (“alternative hydrophilic pathway”). Next to sodium channel blockade, LA exert beneficial effects on pain, stabilize the inflammatory response and the haemostatic system. Inflamed tissue is harder, but not impossible, to anesthetize. Increasing dose or adding a vasoconstrictor, can lead to successful blockade. Systemic toxicity of LA is potentially fatal. When preventative measures are taken, the incidence of cardiac arrest is low. Intralipid has been proposed for resuscitation of systemic LA overdose, and enthusiastically adopted worldwide, even though the mechanism of action is incompletely understood. Intralipid is not an antidote against LA and cannot replace meticulous conduct of regional anaesthesia. All LA are toxic, dependent on dose. The question whether local anaesthetics protect against perioperative tumour progression cannot be answered at this moment, and results from clinical (retrospective) studies are equivocal. Future areas of interest will be design of new subtype-specific sodium channel blockers. At the same time, older LA such as 2-chloroprocaine are reintroduced into the clinical setting. Multimodal perineural analgesia and liposomal bupivacaine may replace catheter techniques in some indications.
Local anaesthetics (LA) are widely used for regional anaesthesia and pain therapy. This review seeks to address 10 essential topics regarding LA use in daily practice.

**General physicochemical properties**

Local anaesthetics are a heterogeneous group of compounds which block voltage-gated sodium channels. Structurally, LA consist of a hydrophobic aromatic group, an amide group, and the connecting intermediary chain. This means that they possess both hydrophobic and -philic properties. According to the type of intermediary chain, LA can be divided into ester-type and amide-type compounds. According to duration of action, LA can be divided into short-acting (e.g., chloroprocaine), intermediate-acting (e.g., mepivacaine, lidocaine), and long-acting (e.g., bupivacaine, ropivacaine) compounds. The metabolism of esters is by plasma cholinesterases and tissue esterases, whereas amides are primarily metabolized hepatically through the mixed-function oxidase system. Conversely, defects in the metabolizing steps lead to higher systemic concentrations of LA. Concerning allergic potential, currently used LA are widely considered to be among the safest perioperative drugs.

All currently used LA are chiral, which means they feature an asymmetric carbon atom and two potential spatial molecular configurations, called enantiomers. The only exception to this rule is lidocaine, which is achiral. The clinical relevance of chirality is that in comparison to racemic mixtures containing both enantiomers, pure enantiomers may offer pharmacologic advantages such as enhanced blockade and decreased toxicity. However, the differences in clinical use when comparing equipotent doses are modest, such that both conventional drugs as bupivacaine, as well as the newer isoforms continue to be in widespread use.

LA have distinct physicochemical properties which determine their mode of action, most notably pKa value, lipophilicity, protein binding, and intrinsic vasoactivity. The main characteristics of clinically used drugs are summarized in Table 1. Importantly, the free systemic fraction of LA, not bound by plasma proteins, is the principal determinant of systemic adverse effects. The rate of
absorption into the systemic circulation depends upon the site of injection. For example, equal plasma concentrations of lidocaine are attained when 300 mg are given intercostally, or 500 mg epidurally, or 1000 mg subcutaneously. A multifactorial approach to choosing safe doses on an individualized basis per patient has been advocated, for which the reader is referred to the comprehensive review by Rosenberg and colleagues.

In plasma, LA are bound to albumine, an abundant protein with weak affinity for LA, and, more importantly, alpha-1-acidic glycoprotein (AAG), less abundant but potent in binding LA. Since AAG is an acute phase protein, synthesis increases postoperatively and after trauma, decreasing free local anaesthetic, and protecting against systemic toxicity. For example, Veering et al. demonstrated that continuous postoperative infusion of epidural bupivacaine leads to increasing levels of total local anaesthetic in the systemic circulation, but at the same time, increasing AAG levels postoperatively bind bupivacaine, resulting in stable levels of systemic free drug. Synthesis of AAG does not mature until one year of age, such that neonates are theoretically at higher risk of elevated free LA plasma levels. Experimental evidence suggests that in pregnancy, protein binding of the more lipophilic bupivacaine is decreased, similarly increasing risk of systemic toxicity.

**Conclusion:** Local anaesthetics are a heterogeneous group of compounds which block voltage-gated sodium channels. Each drug is characterized by distinctive physicochemical properties. The free plasmatic share determines systemic toxicity.

**The primary target: voltage-gated sodium channels**

Local anaesthetics (LA) are primarily characterized by their ability to block voltage-gated sodium channels (VGSC), transmembrane proteins consisting of one main alpha-subunit, linked to one or more beta-subunits. Crystallographic images of sodium channels have become available, improving insight into function and blockade. The alpha subunit of the VGSC constitutes the functional ion channel, and harbours the binding site for LAs. This subunit consists of four domains numbered DI-DIV, each consisting of 6 segments numbered S1-S6 (Figure 1).
contrast, the beta subunits of the VGSC modulate kinetics and voltage dependence of activation and inactivation. The S4 segments are considered the “voltage sensor”, while specific amino acid sequences between segments S5 and S6 mediate the channel’s specificity for sodium.

The binding sites of LA are the S6 segments of domains I, III and IV. Figure 2 gives a schema of the VGSC in cross-section, with (from the outside) the extracellular pore, the selectivity filter, the central cavity, and the innermost activation gate. LA preferably bind to activated and resting channels, because in these states, the activation gate is open, a property described as use-dependent block. Under experimental conditions, binding of the LA to the receptor leads to reversible and concentration-dependent reduction of peak sodium current by modulating the dynamic conformation of the voltage sensing segments S4 across domains of VGSC, and by the creation of a positive charge within the channel’s lumen, directly impeding sodium flux.

Importantly, the family of VGSC consists of at least 10 different subtypes, depending on the gene for the alpha subunit. Dysfunction or mutation has been linked to several pathophysiological states, such as inherited erythromelalgia (syndrome of pain and erythema), paroxysmal extreme pain disorder, congenital insensitivity to pain, cardiac arrhythmias, and epilepsy.

Lastly, drugs other than local anaesthetics can effectively block the neuronal sodium channel. Interestingly, two members of the opiate family, pethidine and buprenorphine, have clinically relevant local anaesthetic properties.

**Conclusion:** Sodium channel block is caused by conformational change and the creation of a positive charge in the channel lumen.

**The three ways for local anaesthetics to block the primary target**

To block the VGSC, classic LA in contemporary use need to attach to a specific binding site on the inner surface of the channel, which they cannot access through the sodium-specific channel itself. Following the “classic hydrophilic” pathway, LA need to traverse the neuronal membrane as uncharged molecules first,
and then conjugate with hydrogen ions and reach the binding site from the cytoplasma (Figure 2).

Another pathway is observed with benzocaine, a permanently uncharged LA, characterized by its low pKa value, and primarily used for topical anaesthesia.23 It reaches the sodium channel directly through the nerve membrane and lateral fenestrations in the channel, a concept supported by recent crystallographic investigations and described as the “hydrophobic pathway”.11

The “opposite compound” of benzocaine is the permanently charged lidocaine derivative, QX-314. Because it is charged, QX-314 will only very slowly cross nerve cell membranes. However, artificial activation of the transient receptor potential vanilloid-1 (TRPV-1) channel allows for the influx of QX-314, and this has been designated the “alternative hydrophilic pathway”.24

Conduction block by LA is first achieved in weakly or non-myelinated fibers (e.g., fibers responsible for the sympathetic nervous system and nociception), whereas myelinated fibers (e.g., motor fibers) are blocked later. However, this effect cannot be explained solely on the basis of different myelin sheath diameters. For example, Huang and co-workers demonstrated that C-type fibers were more resistant to blockade than A-delta- and A-beta-type fibers, which have a larger myelin sheath.25 Rather, it is increasingly appreciated that different neuron populations do not only differ by myelin thickness and size, but also by different patterns of electrophysiologic properties and ion channel composition.26

Conclusion: Different local anaesthetics can reach the LA binding site from the cytoplasmic compartment (“classic hydrophilic pathway”), directly via the lipid membrane (“hydrophobic pathway”), or can enter via large-pore channels (“alternative hydrophilic pathway”).

The secondary targets: from ion channels to G-protein coupled receptors

Next to sodium channel blockade, local anesthetics interact with a wide array of target structures, for example tetrodotoxin-resistant sodium channels, potassium channels, calcium channels, n-methyl-d-aspartate (NMDA) receptors, and G-protein coupled receptors.10

Not surprisingly, systemic local anaesthetics have
been in clinical use for decades. Specific areas of use include administration of lidocaine as class Ib anti-arrhythmic to treat ventricular tachycardia / fibrillation / extra systole, even if recent guidelines limit its use.\textsuperscript{27} Lidocaine has also been proposed as a reserve treatment for refractory status epilepticus.\textsuperscript{28} Similarly, intravenous lidocaine has long been in use for treatment of tinnitus without precise knowledge of the mechanism of action.\textsuperscript{29} Interestingly, when therapeutic systemic doses are exceeded, arrhythmia, seizure and tinnitus are at the same time hallmarks of systemic toxicity.

Systemic LA have a positive stabilizing effect on some physiological systems. Firstly, some studies have described an antinociceptive effect of LA,\textsuperscript{30} while others found no clinically relevant effect.\textsuperscript{31 32} The analgesic effect is potentially caused by lidocaine metabolites modulating glycinergic pathways,\textsuperscript{33} while anti-hyperalgesic effects may, at least in part, be explained by antagonism at the NMDA receptor.\textsuperscript{34} Secondly, lidocaine has for decades been used as a co-anaesthetic, and recent evidence confirms an anaesthetic-sparing effect of lidocaine.\textsuperscript{35} Thirdly, LA have a pronounced anti-inflammatory effect, as they modulate virtually every step of the inflammatory cascade, including leukocyte adhesion to the endothelium, shape change, transendothelial migration, phagocytosis, priming and release of inflammatory mediators.\textsuperscript{36} One potential mechanism underlying the anti-inflammatory effects of LA may be the modulation of G-protein coupled receptor signalling, in particular interference with G-proteins of the Gq/11 family, that predominantly mediate haemostatic and inflammatory signalling, such as the LPA-, TXA2- or PAF-receptor.\textsuperscript{37} However, the complete mechanisms of LA action on these receptors and certain G-protein families remain to be finally determined. Lastly, LA reduce hypercoagulability without inducing a clinically relevant bleeding risk.\textsuperscript{38}

Blockade of potassium channels has been demonstrated for amide-type local anesthetics, contributing to a more intense nerve fibre block,\textsuperscript{39} and explaining symptoms of central nervous system (CNS) excitation such as tinnitus and seizures through depolarization of thalamocortical neurons.\textsuperscript{40} Well-recognized syndromes such as some forms of Long QT syndrome (LQTS) due to potassium channel...
mutation share important pathogenic features with systemic toxicity of local anaesthetics. Another site of local anaesthetic blockade is the nicotinic acetylcholine receptor, a nonselective cation channel. This mechanism of action is thought to contribute to the enhancement of neuromuscular blockade by local anaesthetics, but the clinical importance seems minor.

Alternative effects, however, depend on adequate systemic levels of LA. Classic pharmacokinetic studies carried out on rodent sciatic nerves suggest that only a minor fraction of LA actually participates in nerve block, the majority being absorbed into the systemic circulation. The use of ultrasound-guided regional anaesthesia has led to the administration of very small volumes. For example, Renes and coworkers used approximately a tenth of the dose of LA for interscalene block as compared to the original description by Winnie. These small doses will invariably result in decreased plasma levels of LA, potentially decreasing incidence and severity of systemic toxicity, but at the same time the sharply decreased plasma levels may result in the loss of the above mentioned beneficial effects which are only beginning to be fully appreciated and understood. In the view of the authors, the desire to benefit from systemic effects will need to be weighed against the challenge to find the minimum effective dose for daily clinical practice.

Conclusion: Next to sodium channel blockade, LA exert beneficial effects on pain, stabilize the inflammatory response and the haemostatic system.

Local anaesthetics and inflamed tissue

It has long been accepted that LAs fail in inflamed tissue. However, there is literature that inflamed tissues may be anaesthetized sufficiently, given higher concentrations of LA. For example, Rood and coworkers demonstrated that in dental anaesthesia for inflamed teeth, lidocaine 2% frequently failed to provide a satisfactory block, whereas the success rate of lidocaine 5% was excellent. It is the authors’ opinion that the current viewpoint “inflamatory tissue renders LA inactive” should be adapted to “LA may not function optimally in inflammatory tissue”. Three theories have been put forward to explain decreased function of LA under inflammatory conditions: an acidic shift in tissue pH, increased excitability of
nerves in inflamed tissues, and increased vascularity leading to enhanced absorption. Harris described improved efficacy of LA under inflammatory conditions when injected together with a vasoconstrictor \(^5\).

**Conclusion:** Inflamed tissue is harder, but not impossible, to anesthetize. Increasing dose or adding a vasoconstrictor, can lead to successful blockade.

**Systemic toxicity**

Since the early 1980s, awareness regarding potentially fatal LA overdose led to the introduction of several safety measures, such as incremental administration of LA (including test doses), repeated aspiration tests, compulsory monitoring of the patient, and recommendations regarding maximum dosage. Recent large suggest a low incidence of relevant systemic toxicity. Barrington, in a case series of >20,000 patients, reported an overall incidence of mild signs of local anesthetic systemic toxicity of approximately 1 : 1,000, which was decreased to 1 : 1,600 when ultrasound guidance was used. In the entire case series, one LAST progressed to cardiac arrest.\(^5\) Sites, in a case series of more than 12,000 patients did not report a single case of cardiac arrest.\(^3\)

In general, two forms of LAST can be differentiated: On one hand, “instant” LAST results from intravenous or -arterial injection of LA, and occurs immediately after injection. On the other hand, “slow” LAST results from excessive plasma levels due to overdosing, excessive absorption, reduced metabolism, or reduced plasma protein binding. Slow LAST may occur up to 30 minutes after injection.\(^4\) The initial presentation of slow systemic toxicity will vary depending on the plasma level of free LA.

The classic cascade of systemic toxicity encompasses central nervous system (CNS) symptoms increasing in severity from excitation to seizure to coma. CNS excitation first causes an initial tachycardia and hypertension, while subsequently, cardiac side-effects predominate and lead to progressive circulatory collapse (Figure 3). It should be kept in mind that LA have differential cardiotoxic effects. Substances such as lidocaine and mepivacaine predominantly affect
contractility, whereas ropivacaine, levobupivacaine and bupivacaine are both negatively inotropic and highly arrhythmogenic.\textsuperscript{55}

\textit{Conclusion:} Systemic toxicity of LA is potentially fatal. When preventative measures are taken, the incidence of cardiac arrest is low.

\textbf{Intralipid: critical appraisal}

Treatment of LA-induced systemic toxicity consists of supportive treatment to interrupt seizures and support cardiocirculatory function, and most contemporary guidelines advocate administration of Intralipid\textsuperscript{56,57} which was first shown in 1998 to Intralipid\textsuperscript{®} was first found to decrease the susceptibility of rodents to bupivacaine-induced systemic toxicity.\textsuperscript{58}

Treatment using Intralipid\textsuperscript{®} is thought to induce three changes: first, according to the lipid sink hypothesis, Intralipid\textsuperscript{®} can bind free LA. Secondly, experimental findings suggest Intralipid\textsuperscript{®} to interact with the sodium channel, and thirdly, the lipid emulsion may support mitochondrial metabolism.\textsuperscript{59} Interestingly, the efficacy of Intralipid\textsuperscript{®} to counteract bupivacaine toxicity is dependent on the model used. While Intralipid\textsuperscript{®} has generally shown good effects in rodent models, studies in porcine models, widely used in resuscitation research, generally show no beneficial effects, and the debate which model is more relevant continues.\textsuperscript{50} There exists only one human trial on effects of Intralipid\textsuperscript{®}. Litonius and coworkers infused bupivacaine intravenously, followed by Intralipid\textsuperscript{®}. The authors found reduced context-sensitive half-life of bupivacaine, potentially due to increased tissue distribution, but failed to detect a relevant lipid sink effect.\textsuperscript{61} Therefore, the evidence-base concerning Intralipid\textsuperscript{®} effects remains unclear, and extrapolation of clinically relevant benefits is not straightforward.

Traditionally, bupivacaine has been considered more cardiotoxic based on the smaller dose needed to elicit toxicity as compared to other LA. However, when comparing bupivacaine with, e.g., ropivacaine, it is imperative to consider the 40-50% potency difference. In animal models of toxicity, ropivacaine and bupivacaine are near equipotent in eliciting CNS symptoms.\textsuperscript{62} Nevertheless, even if the toxic threshold is similar for equipotent doses of LA, another consideration is that...
bupivacaine will also remain at the sodium channel longer because of its slower kinetics (slow-in, slow-out).\textsuperscript{63} This needs to be weighed against a possible larger therapeutic effect of Intralipid\textsuperscript{®} in bupivacaine-induced toxicity because in theory, less lipophilic drugs will be affected less by the lipid sink mechanism.\textsuperscript{64}

Recent pharmacokinetic studies have suggested that Intralipid\textsuperscript{®} will decrease the cardiac bupivacaine concentration has been estimated to drop by 11\% within 3 minutes of Intralipid administration, and bupivacaine content of the brain by 18\% within 15 minutes.\textsuperscript{65} Despite the theoretic nature of these findings, they are notable because they underline that Intralipid\textsuperscript{®} reduces, but does not eliminate bupivacaine. Intralipid\textsuperscript{®} should not be considered an antidote the same way that a sufficient dose of protamine will reliably antagonize any amount of heparin. Rather, limited evidence suggests that it will considerably reduce the bupivacaine concentration in target organs, most likely improve metabolism, and potentially have direct beneficial effects at the sodium channel. Intralipid\textsuperscript{®} is a valuable contribution to, but not a substitute for, careful and meticulous conduct of regional anaesthesia.

Conclusion: Intralipid\textsuperscript{®} has been proposed for resuscitation of systemic LA overdose, and enthusiastically adopted worldwide, even though the mechanism of action is incompletely understood. Intralipid\textsuperscript{®} is not an antidote against LA and cannot replace meticulous conduct of regional anaesthesia.

Local anesthetic-induced neuro- and myotoxicity

All local anaesthetics are toxic on virtually any type of tissue. In the clinical setting, neuro- and myotoxicity constitute the main focus of attention.

On neuronal cells, local anaesthetics exhibit dose-dependent toxicity.\textsuperscript{66} The question whether some local anaesthetics are more toxic than others has not been definitively answered and some experimental evidence suggests that equipotent doses of local anaesthetics exhibit the same degree of toxicity,\textsuperscript{66} \textsuperscript{67} whereas other investigations found that lidocaine was more toxic than, e.g., bupivacaine.\textsuperscript{68} \textsuperscript{69} In the classical observational study by Auroy et al., spinal anesthesia performed using lidocaine was associated with more neurological deficits than bupivacaine.\textsuperscript{70} While
the short-term neurological dysfunction after peripheral nerve block is relatively frequent, permanent loss of function is rarely observed.\textsuperscript{71}

Transient neurological syndrome (TNS) typically presents after resolution of a spinal block, and is typically characterized by radicular segmental pain without motor deficit, and spontaneous recovery within 72 hours.\textsuperscript{72} Etiology is multifactorial, and both positioning (lithotomy) and drug (especially lidocaine) are important pathogenic factors.\textsuperscript{73,72}

At the other end of the spectrum, cauda equina syndrome is a generalized destruction of lumbar and sacral nerve fibers, resulting in pain and both sensory and motor deficit, transcending segmental barriers. The incidence has been estimated close to 1 : 10,000\textsuperscript{70,74} and importantly, there are many causes other than LA-induced neurotoxicity, including hematoma, abscess formation, tumours, epidural lipomatosis, and ossifications. Classically, this syndrome was associated with the pooling of hyperbaric lidocaine 5% in the dural sac when repetitively applied via a spinal microcatheter,\textsuperscript{75} lending support to the concept of dose-dependent LA-induced neurotoxicity.

Myotoxicity of local anaesthetics has been appreciated for a long time,\textsuperscript{76} but the clinical relevance is unclear. Most likely, the regenerative potential of muscles and the functional redundancy mask myotoxic effects after most instances of peripheral nerve blockade. Therefore, potential transient myotoxic effects are observed most frequently after regional blockade for eye surgery, where a delicate balance between weak muscles is easily disturbed by myotoxicity.\textsuperscript{77}

Conclusion: All LA are toxic, dependent on dose. Neurotoxicity is rare, but disastrous for the patient when it happens. Myotoxicity is thought to occur frequently, but is only clinically apparent after ocular anesthesia.

Local anaesthetics and tumour recurrence

The perioperative period of tumour surgery has long been recognized as a vulnerable timeframe, during which tumour progression and metastasis is often accelerated.\textsuperscript{78} In small retrospective studies, it was suggested that regional or local anaesthesia may improve patient survival and disease-free interval after cancer
Several experimental studies support this assumption. For example, Deegan and colleagues found that serum taken from patients undergoing tumour surgery under regional anaesthesia halted growth in a tumour cell line \textit{in vitro}.\textsuperscript{81} As a consequence, several large-scale multicentre randomized controlled trials are currently under way to assess beneficial effects of regional anaesthesia in tumour surgery, but they are projected to last until the end of the decade because of long follow-up times.\textsuperscript{82} In the meantime, a number of retrospective analyses has been published, showing heterogeneous effects (Table 2).

The potential mechanisms of anti-metastatic effects can be divided into direct and indirect effects of LA. Direct effects include the interference with tumour-promoting pathways,\textsuperscript{83} changes in the epigenetic signature of tumour cells,\textsuperscript{84}, or direct toxic effects when used for local infiltration.\textsuperscript{80} Indirect effects result from reduction of the perioperative stress response, and the preservation of the immune response.\textsuperscript{85} Since many of these effects can be duplicated using intravenous LA,\textsuperscript{32} another avenue of research may be the employment of systemic LA in the perioperative period of tumour surgery.

We forecast that even if large-scale outcome studies find positive effects, these will most likely not be universal, but limited to specific types of cancer, and possibly, cancer stage. It will be imperative to integrate knowledge obtained in these trials into patient- and tumour-specific strategies to decrease tumour progression during the perioperative period with all available techniques. This will necessitate an overall patient care concept including, for well-described indications, regional anaesthesia.

\textit{Conclusion}: The question whether local anaesthetics protect against perioperative tumour progression cannot be answered at this moment. Results from clinical (retrospective) studies are equivocal.

\textbf{The future of local anaesthetics}

Currently, research efforts are being directed towards the generation of isoform-specific sodium channel blockers, which will allow for the targeted treatment of, e.g., neuropathic pain states.\textsuperscript{86} Still, the idea of targeting of local
anaesthetics into nociceptors is of great potential interest.\textsuperscript{24} Even though the combination of QX-314 and capsaicin does not lend itself to clinical application, the concept is enticing. Substantial research efforts are undertaken to compare effects of intravenous administration of local anaesthetics with regional blockade, leading to new considerations concerning indications of regional anaesthesia.\textsuperscript{87} In this context, it will be essential to define outcome-based patient- and procedure-specific indications for preventive analgesia using either regional anaesthetic or multimodal techniques,\textsuperscript{88} or, in selected patients and indications, both.\textsuperscript{89}

At the same time, “old” local anaesthetics are being rediscovered for new indications. For example, chloroprocaine, prilocaine and articaine have been suggested as novel local anaesthetics for day-case surgery.\textsuperscript{90, 91}

The same way as multimodal systemic analgesia has been adopted for perioperative pain treatment, multimodal perineural analgesia has been advocated. This technique uses a combination of several drugs for peripheral nerve blocks to avoid the need of catheter techniques.\textsuperscript{92}

As an alternative to catheter techniques and adjuvants, the liposomal slow-release formulation of bupivacaine, Exparel\textsuperscript{®}, was recently granted FDA approval for wound infiltration. When used for wound infiltration as part of a multimodal treatment regimen, encouraging results were obtained,\textsuperscript{93} but application for nerve block showed substantial inter-individual variation in block characteristics,\textsuperscript{94} such that further studies are needed to ascertain fitness of Exparel\textsuperscript{®} for regional anaesthesia.

\textit{Conclusion:} Future areas of interest will be design of new subtype-specific sodium channel blockers. At the same time, older LA such as 2-chloroprocaine are reintroduced into the clinical setting. Multimodal perineural analgesia and liposomal bupivacaine may replace catheter techniques in some indications.

The mechanisms of action and access pathways of LA, and their local pharmacokinetics are increasingly understood and appreciated. Only very small amounts of LA actually take part in sodium channel block, while most is absorbed into tissues or the systemic circulation. Systemic LA are responsible for a substantial
Local anesthetics – an introduction

share of beneficial effects of regional anesthesia. The dose of LA administered should be tailored to block site and the individual patient. LA, administered systemically or locally, will remain of paramount importance in perioperative medicine.

Acknowledgments

Presentation: This article is based upon the Refresher Course “Local anesthetics – the essentials” held at the Euroanaesthesia congress, June 2014, Stockholm, Sweden.

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Figure legends

**Figure 1** Spatial configuration of the alpha subunit of the voltage-gated sodium channel. The most frequent mutations related to inherited erythromelalgia,\textsuperscript{17} paroxysmal extreme pain disorder,\textsuperscript{18} congenital insensitivity to pain\textsuperscript{18} are given. Reproduced with permission from Elsevier Publishers: Brain Res Rev 60: 65-83.\textsuperscript{13}

**Figure 2** Crystallographic structure of the sodium channel
(A) Side view of the sodium channel, with (from top) extracellular pore, the selectivity filter, the central cavity, and the innermost activation gate, and (B) Top view of the same structure. Local anaesthetics exist in an equilibrium between the uncharged form B and the charged form BH+, where B can cross the membrane but BH+ is required to bind to the channel. H+ denotes hydrogen ions. Adapted by permission from Macmillan Publishers Ltd: Nature 475: 353-359. Copyright 2011.\textsuperscript{11}

**Figure 3** Sequence of events during local anaesthetic induced systemic toxicity
Increasing systemic concentrations of free local anaesthetic lead to a cascade of central nervous system and cardiac symptoms.
Figure 1
Figure 2
Figure 3
Section 2

Clinical applications
Chapter 2.1

Spinal anaesthesia-induced hypotension

Based on

Lirk P, Haller I, Wong CA

Management of spinal anesthesia-induced hypotension: a European survey

Eur J Anaesthesiol 2013; 29: 452-3
Introduction

Hypotension following administration of spinal anaesthesia for caesarean delivery is common; the reported incidence varies between 50 and 100%.

Several strategies have been suggested to reduce the incidence, or mitigate the severity, of hypotension, such as patient positioning, fluid administration, and use of vasopressors to prevent or correct hypotension. The last decade has seen extensive research efforts to devise the optimal regimen for prevention or treatment of spinal anaesthesia-induced hypotension, including type of intravenous fluid (crystalloid or colloid), timing of fluid administration (before or after initiation of spinal anaesthesia), and choice of vasopressor (epinephrine or phenylephrine). However, the degree to which these new findings have been incorporated into clinical practice is unknown. A survey conducted among American obstetric anaesthesiologists in 2007 found considerable change in clinical practice compared to a UK survey conducted in 1999. Specifically, the use of ephedrine had dropped from 95% to 32%, likely reflecting increased awareness of the potential adverse effects of ephedrine, i.e. tachyarrhythmia and foetal acidosis. There exist no recent data on clinical practice among anaesthesiologists providing care for parturients in Europe.

The aim of the present survey was to determine the clinical practice of spinal anaesthesia for caesarean delivery, focusing on prevention and treatment of post-spinal anaesthesia hypotension among European anaesthesiologists in 2010.

Methods

We developed a web-based survey questionnaire using an online survey engine (www.2ask.at). The initial pilot version was tested by several anaesthesiologists not involved in the study. The survey questions were created by two authors (P.L., I.H.) after reviewing literature about vasopressor and fluid therapy use in obstetric anaesthesia. Following approval by the Scientific Committees of the European Society of Anaesthesiologists (ESA) and the European Society of Regional Anaesthesia and Pain Therapy (ESRA), an e-mail containing an invitation to participate in the survey was distributed to members of the two societies. A total number of 5540 emails were sent, of which 131 were returned.
undelivered, resulting in 5409 contacted members. The invitation email was sent out in May 2010, and the survey remained open for two months. To ensure confidentiality, survey responses did not contain personal or institutional identifying information. Moreover, the invitation emails were distributed by ESA and ESRA headquarters anonymously. No follow-up of non-responders was done. All responses were stored on a secure database and were exported to Microsoft Excel 2003 and SPSS 16.0 for analysis. Plausibility testing was performed and three responses were discarded.

The survey collected demographic data and assessed practitioners’ routine methods for preventing and treating hypotension during spinal anaesthesia, focussing on intravenous fluid administration and vasopressor use. The questionnaire employed multiple branching, allowing responders to loop through a set of multiple-choice questions based on their initial responses to several key questions. The complete survey questionnaire is found in the Appendix.

Results

A total of 5409 email invitations were delivered. We received 351 responses with fully completed questionnaires, which represented an overall response rate of 6.5%. Sixty-seven incomplete questionnaires were excluded from further analysis.

Demographics

Demographic characteristics of the responders are found in Table 1. The responders were distributed among 36 European or Mediterranean countries, with 12 participants (3.4%) from the United States and Australasia. A third of the responders were members of a professional obstetric anaesthesia society (Society for Obstetric Anesthesia and Perinatology or Obstetric Anaesthetists Association). The most common type of anaesthesia for cesaerean delivery was spinal anaesthesia.
Performance of spinal anaesthesia

Details of monitoring and spinal anaesthesia technique are found in Table 2. Blood pressure, heart rate, electrocardiography and pulse oximetry were used by almost all practitioners; other techniques such as bispectral index, fetal heart rate, and urinary output were used by less than 1% of participants. The majority of responders routinely administer oxygen. Spinal anaesthesia was initiated in the sitting position by the majority of anaesthesiologists. The most common spinal anaesthetic was bupivacaine. The use of adjuvants, particularly opioids, was common.

Management of post-spinal anaesthesia hypotension

Details of preventative and treatment measures for spinal anaesthesia-induced hypotension are found in Table 3. Responders estimated that the mean incidence of post-spinal anaesthesia hypotension was 42% (range 1% to 100%). Over 80% of responders routinely administer fluid, most commonly crystalloid, between 500 and 1000 mL. Preload is administered more commonly than coload. Eighty-three percent of responders agreed that patients generally fall into two possible categories: hypotension associated with tachycardia, or hypotension associated with bradycardia (Figure 1). Responders estimated tachycardia occurs more often (63%) than bradycardia (36%). Slightly more than 60% of anaesthesiologists routinely used a vasopressor to prevent hypotension. The most common vasopressor was ephedrine; fewer used phenylephrine and a small minority used another vasopressor. Almost half of the responders always used ephedrine as the first choice vasopressor to treat hypotension, whereas only 14% always used phenylephrine for this indication. Others chose the drug depending on the patient’s heart rate.

Discussion

The main findings of this 2010 survey are that ephedrine was still routinely used by more than 70% of survey responders practicing primarily in Europe, for the treatment of hypotension associated with spinal anaesthesia in women undergoing
caesarean delivery; however, more than 40% also use phenylephrine. One quarter of responders used either ephedrine or phenylephrine, depending on heart rate and / or blood pressure.

Response rate and demographics

A limitation of our results is our low response rate. The low rate most likely represents the anonymity with which participants were contacted, and the lack of follow-up. Hazard Munro postulated that that the minimal number of survey responses required for survey validity is equal to the number of questions times ten; therefore the current 28- question survey required at least 300 response, a number which was exceeded in the present study. The preferred method to increase response rate would be to decrease sample size while increasing efforts to contact non-responders. Unfortunately, due to the distribution of the invitation email by the two anaesthesiology societies, this technique was not possible in the present study. As in all surveys, nonresponder bias may constitute a source of error. However, the characteristics of the test persons in our study compare well with those of previous surveys, with a slight majority working in community hospitals, and slightly less than half practicing academic anaesthesiology. Also, our responders were distributed over different European and Mediterranean countries, therefore meeting our aim of surveying predominantly European anaesthesiologists. The share of caesarean deliveries among overall deliveries was reported by responders to be approximately 25%, corresponding well with previous investigations. Most responders performed elective caesarean delivery under spinal anaesthesia or other neuraxial anaesthesia modalities (epidural, combined spinal-epidural), although 16.2% of caesarean deliveries were estimated to occur under general anaesthesia. These numbers correspond with recent European surveys on use of general anaesthesia for caesarean deliveries. In Germany the rate of general anesthesia decreased from 71% in 1998 to 27% in 2005, in Italy the rate was 34% in 2007, and in Israel the rate was 15% in 2010.

Finally, the response rate may have been negatively affected by the specific nature of the survey. Because almost 80% of responders perform obstetric
anaesthesia routinely, we surmise that the survey was completed by a group of anaesthesiologists with a dedicated and collective interest in obstetric anaesthesiology.

Performance of spinal anaesthesia

The practice of administering oxygen to parturients in women with normal oxygen saturation is controversial, although a majority of the survey’s responders do so routinely. High inspired oxygen concentrations during elective caesarean delivery under spinal anaesthesia have been controversially discussed to cause oxygen free radical activity in both mother and foetus.\textsuperscript{14, 15} Potential adverse effects of high neonatal oxygen concentrations have been demonstrated in several neonatal diseases such as bronchopulmonary dysplasia, and retinopathy of prematurity.\textsuperscript{14} Historically, it has been thought that supplementary oxygen delivered to the mother might protect the foetus against desaturation in the event of prolonged incision-to-delivery interval during elective caesarean section, but this was disproved by Khaw and colleagues.\textsuperscript{16} Administration of 60% oxygen to parturients undergoing emergency caesarean section under spinal anesthesia resulted in improved oxygenation indices in the fetus, without increase in lipid peroxidation, and similar outcome.\textsuperscript{17} The clinical relevance of these findings remains unclear, but the routine administration of oxygen to every parturient irrespective of peripheral oxygen saturation is not supported by literature. Some anaesthesiologists may be administering oxygen solely because doing so allows simultaneous measurement of end-tidal carbon dioxide.

The majority of responders used bupivacaine as the local anaesthetic, combined with fentanyl, sufentanil, or morphine. Very few used clonidine or epinephrine, while 13% did not use any adjuvants. Interestingly, the majority of responders base the anaesthetic dose on clinical experience or body-mass or height nomograms, while approximately 20% use the same dose of local anaesthetics in all patients. The latter practice is supported by recent investigations which found no relationship between body mass index (BMI) and dose-response for spinal anaesthesia for elective caesarean delivery.\textsuperscript{18, 19} Hence, current literature does not support altering the local anaesthetic dose based on body habitus.
Management of post-spinal anaesthesia hypotension

Many responders were not aware of guidelines for the prevention and treatment of hypotension. The reported incidence of post-spinal hypotension varies widely, with a range of 7% to 74% in prospective studies, depending on the definition of hypotension and method of measurement.20 Wide heterogeneity exists in the definition of hypotension, and consequently, the treatment goal. The most common definitions of hypotension after spinal anaesthesia for caesarean delivery are “below 80% of baseline” or a combined “below 80% of baseline or 100 mmHg systolic”.20

In our survey, the target blood pressure goal among clinicians varied widely. In a recent North American survey by Allen et al., the most common threshold for treatment of hypotension was less than 20% of baseline pressure, whereas 13% tried to maintain BP at baseline level.4 In survey of British obstetric anaesthesiologists a systolic blood pressure of 100 mmHg was defined as threshold for vasopressor administration by 44% of responders; 41% chose a threshold of 90 mmHg.5 Recent data suggest that maintaining blood pressure close to baseline compared to 80% baseline is associated with higher umbilical artery pH values.21 In our survey, the overwhelming majority of responders tried to maintain blood pressure at more than 80% of baseline level, which is in keeping with previous investigations; less than 5% aimed to keep pressure at baseline.

Research has attempted to determine risk factors for post-spinal hypotension. Retrospective analysis suggests chronic alcohol consumption, history of hypertension, increased body mass index, sensory block height and urgency of surgery as independent risk factors for post-spinal hypotension.22 Recently, body mass index, sensory block level and older maternal age were identified as independent risk factors for maternal hypotension, but inclusion of these factors into a prospective risk assessment showed moderate sensitivity23 and despite preventive measures, clinicians should continue to anticipate hypotension in every parturient in which spinal anaesthesia is performed for caesarean delivery.
Adverse effects of hypotension are both maternal (syncope, nausea and vomiting) and foetal/neonatal (placental hypoperfusion, hypoxia, acidosis).\textsuperscript{2} In a retrospective study of 919 mother-infant pairs, maternal hypotension was not found to predict perinatal complications if promptly treated,\textsuperscript{24} but prolonged hypotension is associated with foetal acidosis \textsuperscript{25} and may cause maternal and foetal morbidity.\textsuperscript{26}

The wide range of responses of our responders estimating the incidence of post-spinal hypotension (1\% to 100\%), may reflect individual anaesthesiologists’ experiences with various preventive strategies, as well as prophylactic versus treatment use of vasopressors. Maintenance of blood pressure was most often achieved by a combination of fluid and vasopressor therapy. The crystalloid volume range reported by responders was consistent with the volumes reported in most clinical trials.\textsuperscript{27}

Fluid loading appears insufficient on its own to prevent or treat hypotension following spinal anesthesia in parturients. Most responders administered crystalloid as a preload despite evidence suggesting that this practice is ineffective.\textsuperscript{2, 28-30} Measurements of cardiac output during caesarean delivery show that the beneficial effects of a pre-load fluid bolus are not sufficient to maintain hemodynamic stability after the initiation of spinal anaesthesia.\textsuperscript{31} It is likely that the increases in cardiac output that result from fluid loading are insufficient to compensate for the decrease in systemic vascular resistance caused by high-thoracic neuroblockade.\textsuperscript{31} Moreover, rapid pre- or coload may lead to activation of atrial natriuretic peptide in response to increased intravascular volume, thereby counteracting desired volume-expanding effects in both healthy and pre-eclamptic parturients.\textsuperscript{32, 33}

Colloid is more reliable than crystalloid in preventing hypotension in a systematic review,\textsuperscript{34} but a 2010 meta-analysis concluded that the incidence of maternal hypotension remains high, no matter whether crystalloid or colloid is administered as a preload or coload.\textsuperscript{27} Since colloids are associated with a small but measurable risk of allergic reactions,\textsuperscript{34} choice of fluid must weigh the small risk of allergic reactions (estimated at 0.03\%\textsuperscript{35}) associated with colloid administration against its value in preventing hypotension following spinal anaesthesia.\textsuperscript{34}
Spinal anaesthesia-induced hypotension

In a recent trial, colloid pre-load was found to increase cardiac output transiently for the first five minutes after spinal anesthesia, but there was no difference in vasopressor requirements or blood pressure changes between colloid preload and coload. The rationale behind fluid pre- and co-load is to counteract decreased cardiac output due to decreased venous return following spinal anesthesia. However, recent evidence emphasizes the importance of changes in systemic vascular resistance in post-spinal anesthesia hypotension, with the possible exception of patients who are hypotensive and bradycardic after spinal anesthesia. This latter group of patients should preferentially be treated using fluid, ephedrine or epinephrine, and possibly anticholinergic agents. The proportion of bradycardic patients after spinal anesthesia was estimated by our responders, on average, to comprise around a third of patients, whereas a much lower incidence was reported in recent trials and a recent review of hemodynamic changes following spinal anaesthesia for caesarean section described the incidence as low.

Despite preventive measures and fluid co-load, hypotension after spinal anaesthesia is common. In a recent study, the combination of prophylactic phenylephrine and rapid crystalloid cohydration was found to be effective in preventing spinal-induced hypotension for caesarean delivery. Ngan Kee et al. postulated that cohydration better coincides with peak sympathectomy effect of spinal anaesthesia, and promotes faster circulation of vasopressor. This latest study achieved a very low incidence of maternal hypotension, albeit at the cost of overcorrecting blood pressure in a substantial share of patients.

Perioperative measurements of cardiac output have shown that the major changes induced by spinal anaesthesia are a decrease in systemic vascular resistance accompanied by a compensatory increase in cardiac output. This pathogenic mechanism is directly counteracted by phenylephrine, which increases systemic vascular resistance, and decreases cardiac output. Ephedrine, in contrast, increases heart rate and (to a lesser extent) stroke volume and therefore cardiac output, but is often not potent enough to correct changes in vascular resistance.
Whether ephedrine of phenylephrine is the vasopressor of choice for post-spinal hypotension in elective caesarean delivery remains controversial. However, recent editorialists suggest that based on current evidence, phenylephrine is the drug of choice. Ephedrine has been associated with foetal acidemia, and both the spinal anaesthesia - delivery interval and total ephedrine dose are positively correlated with neonatal academia. In a secondary analysis of the EPIPAGE trial data, neonatal mortality of preterm infants was higher after spinal than general or epidural anaesthesia; it has been hypothesized that the widespread use of ephedrine may be responsible for this finding. The mechanism of action of ephedrine’s potential adverse effects has not been clearly elucidated. Ephedrine crosses the placenta to a greater extent than phenylephrine, and it may exert beta-adrenergic mediated stimulatory effects on foetal metabolism.

Accumulating evidence the phenylephrine is superior to ephedrine appears to have resulted in an increased use of phenylephrine for the prevention and treatment of hypotension in the past 11 years. Results of surveys in North American and the United Kingdom in 1999 and 2007 suggest that the use of phenylephrine in the obstetric population is increasing. In the 1999 survey, ephedrine was used by 95% of responders. In contrast, a 2007 survey found 23% and 26% of responders used phenylephrine for the prevention and treatment of hypotension, respectively. In the same survey, 40% of anaesthesiologists used either agent based upon heart rate. In our survey, 26% of responders used either agent based upon heart rate. This is also in concordance with recent literature, which recommends that phenylephrine may be the better choice in situations when hypotension coincides with tachycardia, whereas ephedrine may be better suited in situations when hypotension coincides with bradycardia. However, in light of the strong evidence to support routine use of phenylephrine, we note the relatively large number of responders who still always use ephedrine as the first choice, while a much smaller number of responders reported the exclusive use phenylephrine.

In conclusion, we describe results from a 2010 European survey investigating contemporary clinical practice in the management of hypotension following spinal anaesthesia for caesarean delivery. In contrast to previous surveys,
Spinal anaesthesia-induced hypotension

we found increased use of phenylephrine to treat hypotension, in keeping with a growing evidence-base suggesting its side-effect profile is superior to that of ephedrine in most cases. Recent literature supports the use of phenylephrine as the first-line drug to treat most patients with hypotension. Nevertheless, many clinicians appear to continue to use ephedrine routinely.

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Table 1. Responder demographic characteristics.

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</table>

<table>
<thead>
<tr>
<th>Obstetric anaesthesia practice</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Full time</td>
<td>10%</td>
</tr>
<tr>
<td>Routinely</td>
<td>78%</td>
</tr>
<tr>
<td>Rarely</td>
<td>12%</td>
</tr>
</tbody>
</table>
**Table 2. Characteristics of spinal anaesthesia.**

<table>
<thead>
<tr>
<th>Administration of oxygen yes/no</th>
<th>60%/40%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local anesthetic (multiple answers possible)</strong></td>
<td></td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>89%</td>
</tr>
<tr>
<td>Levobupivacaine</td>
<td>7%</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>6%</td>
</tr>
<tr>
<td>Lidocaine/Mepivacaine</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Additives (multiple answers possible)</strong></td>
<td></td>
</tr>
<tr>
<td>Fentanyl</td>
<td>48%</td>
</tr>
<tr>
<td>Sufentanil</td>
<td>29%</td>
</tr>
<tr>
<td>Morphine</td>
<td>21%</td>
</tr>
<tr>
<td>Clonidine</td>
<td>1%</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Dehydrobenzperidol</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Pethidine, Diamorphine</td>
<td>&lt; 1%</td>
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</tbody>
</table>
Table 3. Hemodynamic variables and treatment

<table>
<thead>
<tr>
<th>Blood pressure goal</th>
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</tr>
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<tbody>
<tr>
<td>at/above baseline</td>
<td>4%</td>
</tr>
<tr>
<td>above 90% of baseline</td>
<td>40%</td>
</tr>
<tr>
<td>above 80% of baseline</td>
<td>54%</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Fluid pre-load or co-load (multiple answers possible)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid preload</td>
<td>85%</td>
</tr>
<tr>
<td>Crystalloid fluid preload</td>
<td>65%</td>
</tr>
<tr>
<td>Colloid fluid preload</td>
<td>27%</td>
</tr>
<tr>
<td>Fluid co-load</td>
<td>39%</td>
</tr>
<tr>
<td>Fluid volume 500-1000 ml</td>
<td>71%</td>
</tr>
<tr>
<td>Fluid volume 1000-1500 ml</td>
<td>16%</td>
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</table>

<table>
<thead>
<tr>
<th>Preventive vasopressor (multiple answers possible)</th>
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<tbody>
<tr>
<td>Routinely used</td>
<td>63%</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>72%</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>46%</td>
</tr>
<tr>
<td>Other</td>
<td>9%</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Therapeutic vasopressor</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Always ephedrine</td>
<td>48%</td>
</tr>
<tr>
<td>Always phenylephrine</td>
<td>14%</td>
</tr>
<tr>
<td>Phenylephrine 1st line, ephedrine 2nd line</td>
<td>4%</td>
</tr>
<tr>
<td>Ephedrine 1st line, phenylephrine 2nd line</td>
<td>9%</td>
</tr>
<tr>
<td>Choice based upon heart rate</td>
<td>26%</td>
</tr>
<tr>
<td>Others: metaraminol, theodrenaline, noradrenaline</td>
<td></td>
</tr>
</tbody>
</table>
Pulmonary effects of spinal anesthesia

Based on


Pulmonary effects of bupivacaine, ropivacaine, and levobupivacaine in parturients undergoing spinal anaesthesia for elective caesarean delivery: a randomised controlled study

Chapter 2.2

Introduction

Intrathecal anaesthesia has replaced general as the first-line method to provide anaesthesia for elective caesarean delivery.\(^1\) The main reason for the popularity of spinal anaesthesia is that general anaesthesia may be associated with complications in airway management such as aspiration or failure to achieve tracheal intubation.\(^1\) It is unclear whether spinal anaesthesia carries a risk of significant respiratory deterioration due to motor block of respiratory muscles,\(^2\) \(^3\) since caesarean delivery necessitates a spinal block extending as far cephalad as the fourth thoracic segmental nerve.\(^3\) Motor blockade from the lumbar to the thoracic nerves temporarily deactivates some muscles that contribute to respiration, including the intercostal muscles\(^4\) and the abdominal wall muscles.\(^5\)

In clinical practice, bupivacaine is the most widely used local anaesthetic for elective caesarean delivery.\(^6\) \(^7\) Numerous publications have reported that spinal anaesthesia using bupivacaine significantly decreases dynamic pulmonary function parameters in the parturient.\(^2\) \(^3\) \(^5\) \(^8\) It has been suggested that the newer local anaesthetics ropivacaine and levobupivacaine do not cause the same degree of motor block,\(^9\) \(^11\) but the pulmonary effects of these drugs when used for spinal anaesthesia are unclear. For example, epidural and intrathecal levobupivacaine and ropivacaine were shown \textit{in vivo} to elicit less motor block than bupivacaine.\(^12\) Recently, intrathecal bupivacaine was shown in parturients to have a higher potency for motor block than levobupivacaine and ropivacaine.\(^13\) Therefore, the pulmonary effects of ropivacaine and levobupivacaine may be less pronounced than those of bupivacaine.

The aim of the present study was to compare the performance of bupivacaine 10 mg, ropivacaine 20 mg, and levobupivacaine 10 mg for spinal anaesthesia in parturients undergoing elective caesarean delivery. As relevant endpoints, we chose to investigate dynamic maternal pulmonary function parameters and neonatal indices. Our working hypothesis was that pulmonary function would be attenuated by all three local anaesthetics.
Methods

In a single-blind study, 48 otherwise healthy parturients scheduled to undergo elective caesarean section were enrolled and randomised to receive bupivacaine, ropivacaine or levobupivacaine. Randomization of patients into the three treatment groups was performed according to a preset randomization list disclosed from sealed opaque envelopes before spinal anesthesia. Exclusion criteria were pre-existing maternal pulmonary or cardiac disease, thoracic malformation, non-singleton pregnancy, signs of fetal compromise and a body mass index (BMI) $>35 \text{ kg/m}^2$, indicating severe obesity.\textsuperscript{14} The study was approved by the Ethics Committee of the Medical University, Innsbruck (Protocol No. UN2195_ZEK), Austria, and registered with the European Union Clinical Trials Database (EudraCT No. 2004-004649-17). Written informed consent was obtained from all patients. Patients were blinded to the study drug, but the investigator and anaesthesiologist administering spinal anaesthesia were not.

Premedication and spinal anaesthesia

All parturients were preloaded intravenously with 1000 mL of crystalloid fluid (Ringer’s lactate) and received famotidine 20 mg before transfer to the operating room. Non-invasive blood pressure, pulse oximetry and electrocardiogram were monitored. Spinal anaesthesia was performed in the sitting position between the second and fourth lumbar vertebrae using a 25-gauge Sprotte pencil-point needle (Smith, Brisbane, Australia). Patients were randomised to receive 0.5% bupivacaine 10 mg (Curasan, Kleinostheim, Germany), 1% ropivacaine 20 mg (Astra Zeneca, Södertälje, Sweden), or 0.5% levobupivacaine 10 mg (Abbott, Campoverde, Italy). Fentanyl 15 µg (Torrex Pharma, Vienna, Austria) was added to the local anaesthetic in all groups. Sensory block was tested every minute by pin prick in the mid-clavicular line, testing for discrimination between sharp and blunt stimulus, until a stable sensory block level was reached. The lowest mean arterial pressure and vasopressor requirements following spinal anaesthesia were recorded.
Measurement of pulmonary function

When the sensory block had stabilized, dynamic pulmonary function indices were measured using an EasyOne spirometer (ndd, Zurich, Switzerland) before spinal anaesthesia, and when a stable sensory level was achieved. The spirometer used requires several measurements tested for consistency before the best value is accepted as a result. Internal quality control software detects both implausible spirometric results and intra-test variability. Accepted results require at least two measurements of forced expiratory volume during the first second of exhalation (FEV1) or forced vital capacity (FVC) to vary no more than 200 mL. A single investigator (NK), experienced in performing spirometric measurements, recorded all pulmonary function indices; quality control and tabulation of measured data was performed blinded to group allocation. Pulmonary function tests took approximately 2 minutes to perform.

The evening before surgery, the patients were instructed in the use of the spirometer to simulate testing. Baseline measurements were obtained on the day of surgery in the 15° left lateral tilt position. To explore the possibility that the 15° left tilt and supine positions would yield different pulmonary function test results, measurements were obtained in both positions in eight patients. As there were no significant differences in pulmonary function tests, baseline results were pooled. Pulmonary function following spinal anaesthesia were measured in the 15° left tilt position. Caesarean delivery was conducted after measurements were completed.

The main outcome variables were FVC, FEV₁, and peak expiratory flow rate (PEFR). Secondary outcome variables were fetal blood gases and Apgar score. Independent factors included maternal characteristics (age, BMI, smoking status, gestational age) and perioperative data (site of puncture, time for the block to reach T4, duration of surgery, time to delivery).
Statistical analyses

Power calculation based on previous investigations of spinal anaesthesia for caesarean delivery\(^3\) assumed a mean change in FEV\(_1\) of 15-20%. Therefore, 16 test parturients per group were predicted as being necessary to detect a statistically significant alteration in function tests with a power of 85%. The primary purpose of the study was to evaluate if any of the three local anaesthetic agents cause a 15-20% decrease in FEV\(_1\) in parturients undergoing spinal anaesthesia; as such, the power analysis was not designed to indicate differences between the three agents. Normal distribution of data was checked by the Kolmogorov-Smirnov test. The time course of the main variables was tested using analysis of variance (ANOVA) with Bonferroni’s post hoc correction. Secondary variables were tested using the \(\chi^2\) test for qualitative, and ANOVA for quantitative data. \(P\) values <0.05 were considered statistically significant. The statistical null hypothesis was that pulmonary function would not be attenuated by any of the three local anaesthetics. Data were analysed using SPSS version 12.0, Chicago, IL.
Results

Demographics. The 48 parturients were between 22 and 43 years of age. There were 16 patients in each group. There were no significant differences in mean age, BMI, gestation age, smoking status or number of previous pregnancies or cesarean sections between the three groups (Table 1).

Spinal anaesthesia and surgery. The documented site of puncture varied between L2-3 (n=25) and L3-4 (n=23) but was not different between groups. The upper level of block of pin-prick sensation and the time to reach T4 did not differ between the three groups (Table 1). All caesarean deliveries were performed under spinal anaesthesia, without the need for conversion to general anaesthesia. There were no differences in duration of surgery, time to delivery after incision, lowest recorded mean arterial pressure or numbers of patients requiring vasopressors following spinal anaesthesia (Table 1).

Maternal pulmonary function tests. At baseline, the values for FVC, FEV1, and PEFR were normally distributed, and mean values for the three study groups did not differ significantly (Table 2). Forced vital capacity was decreased in the bupivacaine and ropivacaine groups but not in the levobupivacaine group. Forced expiratory volume during the first second was not decreased in any group. Peak expiratory flow rate was decreased in the ropivacaine and levobupivacaine groups. There were no significant differences in pulmonary function indices after spinal anaesthesia between the groups. To examine whether the level of spinal block would influence maternal lung function, all patients were pooled by block level, comparing the subset of patients in whom the block spread to no higher than T4 vs. higher than T4. The maternal lung function parameters after spinal anaesthesia were not different between these two groups (Table 2).

Neonatal status: there were no significant differences in neonatal outcome measures between the groups (Table 3).
Pulmonary effects of spinal anesthesia

Discussion

The effects of neuraxial bupivacaine on pulmonary function have repeatedly been measured, but comparative data for ropivacaine and levobupivacaine have not hitherto been available. We found a significant reduction in selected lung function parameters after intrathecal bupivacaine 10 mg, ropivacaine 20 mg and levobupivacaine 10 mg, all with fentanyl 15 µg, among patients about to undergo elective caesarean delivery. These reductions were in the region of 3-6% for FVC and 6-13% for PEFR. However, the clinical importance of this finding remains unclear. No difference was found between bupivacaine, ropivacaine and levobupivacaine in pulmonary function variables, Apgar scores or umbilical artery pH.

The propensity of ropivacaine and levobupivacaine to cause motor block is thought to be less than that of bupivacaine. One reason for this differential block may be preferential blockade of sodium channels specific for nociceptive neurones by ropivacaine. A recent investigation in parturients undergoing elective caesarean section found that intrathecal bupivacaine, levobupivacaine, and ropivacaine had high, intermediate, and low potency for motor block, respectively. In a similar study, intrathecal ropivacaine was shown to cause less intense and shorter-lasting motor block than bupivacaine. However, Camorcia et al. postulated that the differences in motor block among local anaesthetics were less distinctive when used for spinal than for epidural anaesthesia.

Caesarean delivery necessitates a block to pin-prick or cold extending as far cephalad as the fourth thoracic segmental nerve. Since this blockade may affect some of the intercostal muscles, a substantial share of respiratory capability may be deactivated during spinal anaesthesia. Intercostal muscles play multifaceted roles even during physiologic inspiration and expiration. Nevertheless, Egbert et al. and Askrog et al. failed to detect clinically significant alterations in the capacity to initiate a forceful cough, presumably because of functional compensation by the diaphragm. In addition to the diaphragm, and in contrast to respiration at rest, additional muscles are required for forceful inspiration (i.e. sternocleidomastoid, scalenus, external intercostals) and expiration (i.e. internal intercostals, abdominal
Intercostal muscles after spinal blockade seem to play a minimal role in overall lung function. In our study, results for the subset of patients in whom the block extended no higher than T4 were not significantly different from those for patients in whom the block extended beyond T4. Similarly, Arai et al. recently found that maternal pulmonary function was not significantly different when comparing patients with block level of T1-T2 and T4. It remains unclear whether compensation by the diaphragm is the only factor maintaining forceful expiration in the presence of partially blocked intercostal muscles. Even patients with spinal cord injury involving the mid to lower cervical cord may be able to augment expiration using accessory muscles of respiration.

The concentrations of drugs chosen for this clinical trial were extrapolated from previous investigations, which found a relative anaesthetic potency between bupivacaine and ropivacaine of 2:1 and between bupivacaine and levobupivacaine of 1:1. Therefore, we chose final doses for bupivacaine 10 mg, levobupivacaine 10 mg, and ropivacaine 20 mg. However, in studies published after our study was initiated, different relative potencies were found. For example, Parpaglioni et al. found that the potency ratio between levobupivacaine and ropivacaine was 1:0.74, and not 1:0.5 as assumed in our study. Moreover, Camorcia et al. reported the relative ED₅₀ for motor block after intrathecal administration for ropivacaine:bupivacaine 0.59 and for levobupivacaine:bupivacaine 0.71, so respiratory impairment with ropivacaine might have been less than with the other two agents in our study if a more nearly equipotent dose had been used.

The addition of opioid (fentanyl 15 µg) to all local anaesthetic agents in our study could have theoretically altered the block characteristics and motor function. However, previous investigation in patients undergoing transurethral resection of the prostate demonstrated that lung function was not altered when fentanyl was added to bupivacaine for spinal anesthesia. Although fentanyl might reduce respiratory drive, it would be unlikely to affect capacity.

The dynamic pulmonary function tests were affected to different degrees by spinal anaesthesia. More specifically, FEV₁ did not decrease significantly in any of the study groups, which is consistent in other studies with bupivacaine. By
contrast, Kelly et al. found significant decreases in FEV\textsubscript{1} after spinal anaesthesia,\textsuperscript{3} but their results could have been confounded by an open abdomen, which has been previously shown to substantially influence pulmonary function.\textsuperscript{8}

FVC was negatively influenced by spinal anaesthesia using bupivacaine and ropivacaine, which is in accord with previous investigations.\textsuperscript{8,3} However, Harrop-Griffiths et al.\textsuperscript{2} and Arai et al.\textsuperscript{6} found no significant alterations in FVC after bupivacaine spinal anaesthesia. Possible explanations for these differences include a 12% greater BMI in our patients than among those studied by Arai and colleagues;\textsuperscript{6} the higher BMI appears to predispose patients undergoing spinal anaesthesia to more severe reductions in lung function.\textsuperscript{24,25}

Finally, PEFR decreased significantly with ropivacaine and levobupivacaine, but not with bupivacaine. While Conn et al.\textsuperscript{8} did not detect a significant deterioration of PEFR after spinal anaesthesia with bupivacaine for caesarean delivery, Harrop-Griffiths et al.\textsuperscript{2} and Kelly et al.\textsuperscript{3} reported statistically significant decreases. This disparity may be explained by different doses of bupivacaine: Harrop-Griffiths and Kelly both gave a dose of 12.5 mg. In addition, Kelly measured pulmonary function before spinal anaesthesia, and during surgery, when the abdomen was open, which may further compromise ventilation.\textsuperscript{5}

An important secondary endpoint was the analyses of Apgar scores and umbilical blood gases. In accordance with previous literature,\textsuperscript{26} no significant differences were found. To exclude the potential confounding of these results by hypotension or vasopressor administration, the lowest measured mean arterial pressure following spinal anaesthesia, and vasopressor requirements were recorded. These values were not significantly different between the groups.

Some limitations of our study should be briefly addressed. Firstly, spirometric testing depends to a large extent upon the compliance of the test person and adherence to the experimental protocol may have been difficult during the very stressful moments immediately before delivery. We sought to minimize this confounding variable by preoperative instruction, including hands-on practice with the spirometry device.\textsuperscript{2,3,8} Secondly, not all baseline measurements were taken in the same position (supine vs. 15° left tilt). Even though we performed statistical testing
to assure that these values were not different, we cannot exclude a small influence upon our results. Thirdly, our power analysis was designed to test if the groups achieved a certain decrease in pulmonary function tests, but not to evaluate differences between the groups. Because clinically significant reductions in pulmonary function were not observed in any of the groups, it seems highly unlikely that significant differences would be found between the three groups. Finally, although the present study was randomised and controlled, organisational problems precluded the use of a double-blind technique. Nevertheless, we believe that results from our study are valid and reproducible because the spirometric device required several recordings tested for consistency before computing pulmonary function indices.

The results of our study suggest that spinal anaesthesia with bupivacaine, ropivacaine, or levobupivacaine, all with fentanyl 15 µg, resulted in comparable maternal lung function (FVC, FEV\textsubscript{1}, PEFR), and neonatal outcomes. Based on our findings, the three investigated local anaesthetics may be equally well suited for spinal anaesthesia for elective caesarean delivery.

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20 McDonald SB, Liu SS, Kopacz DJ, Stephenson CA. Hyperbaric spinal ropivacaine: a comparison to bupivacaine in volunteers. *Anesthesiology* 1999; 90: 971-7
21 Alley EA, Kopacz DJ, McDonald SB, Liu SS. Hyperbaric spinal levobupivacaine: a comparison to racemic bupivacaine in volunteers. *Anesth Analg* 2002; 94: 188-93
<table>
<thead>
<tr>
<th></th>
<th>Bupivacaine (n=16)</th>
<th>Ropivacaine (n=16)</th>
<th>Levobupi (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.9 ± 4.0</td>
<td>32.2 ± 5.1</td>
<td>33.9 ± 4.4</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>27.0 ± 3.6</td>
<td>27.5 ± 3.7</td>
<td>28.1 ± 4.9</td>
</tr>
<tr>
<td>Smokers</td>
<td>1/16</td>
<td>5/16</td>
<td>2/16</td>
</tr>
<tr>
<td>Gestation (weeks)</td>
<td>38.6 ± 1.0</td>
<td>38.4 ± 1.0</td>
<td>38.2 ± 0.8</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0-4)</td>
<td>1 (0-4)</td>
<td>1 (0-4)</td>
</tr>
<tr>
<td>Previous caesarean sections</td>
<td>1 (0-3)</td>
<td>1 (0-2)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>Time to T4 (min)</td>
<td>6.3 ± 2.0</td>
<td>6.4 ± 1.7</td>
<td>6.7 ± 2.3</td>
</tr>
<tr>
<td>Time from incision to delivery (min)</td>
<td>9.8 ± 3.7</td>
<td>7.3 ± 3.0</td>
<td>8.7 ± 4.0</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>45.3 ± 12.5</td>
<td>46.5 ± 15.4</td>
<td>50.6 ± 12.8</td>
</tr>
<tr>
<td>Lowest mean arterial pressure (mmHg)</td>
<td>68.5 ± 10.6</td>
<td>65.7 ± 9.7</td>
<td>65.8 ± 11.4</td>
</tr>
<tr>
<td>Number requiring vasopressor</td>
<td>13</td>
<td>14</td>
<td>12</td>
</tr>
</tbody>
</table>

Values mean ± SD, median [range] or number (range). No significant differences between groups.
Table 2. Maternal pulmonary function tests before and after spinal anaesthesia

<table>
<thead>
<tr>
<th></th>
<th>FVC (L)</th>
<th>FEV₁ (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>3.6 ± 0.5</td>
<td>3.5 ± 0.4*</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>3.2 ± 0.4</td>
<td>3.1 ± 0.5*</td>
</tr>
<tr>
<td>Levobupivacaine</td>
<td>3.6 ± 0.5</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>Block lower than T4</td>
<td>3.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Block higher than T4</td>
<td>3.4 ± 0.4</td>
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<table>
<thead>
<tr>
<th></th>
<th>PEFR (L/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>6.2 ± 1.2</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>5.5 ± 1.5</td>
</tr>
<tr>
<td>Levobupivacaine</td>
<td>6.0 ± 1.1</td>
</tr>
<tr>
<td>Block lower than T4</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>Block higher than T4</td>
<td>3.4 ± 0.4</td>
</tr>
</tbody>
</table>

*significantly different from before: \( P <0.05 \) by \( t \) test, NS by ANOVA

** significantly different from before: \( P <0.01 \) by \( t \) test, NS by ANOVA
<table>
<thead>
<tr>
<th></th>
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<th>Ropivacaine</th>
<th>Levobupivacaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apgar scores 1/5/10 min</td>
<td>9/10/10</td>
<td>9/9/10</td>
<td>9/9/10</td>
</tr>
<tr>
<td>UA pH</td>
<td>7.3 ± 0.1</td>
<td>7.3 ± 0.0</td>
<td>7.3 ± 0.1</td>
</tr>
</tbody>
</table>

Data are median or mean ± SD
Failed epidural – causes and management

Based on

Hermanides J, Hollmann MW, Stevens MF, Lirk P

Failed epidural: causes and management

Br J Anaesth 2012; 109: 144-54
**Introduction**

In contrast to the subjective experience of many anaesthesiologists, failure of epidural anaesthesia and analgesia is a frequent clinical problem (feedback error). Current estimates concerning the incidence of failed epidurals are hampered by lack of a uniform outcome parameter. The definitions given cover a spectrum ranging from *insufficient analgesia* to *catheter dislodgement* to *any reason for early discontinuation of epidural analgesia* (Table 1). In a heterogeneous cohort of 2140 surgical patients, failure rates of 32% for the thoracic, and 27% for the lumbar epidural were described.\(^1\) Of note, active management of insufficient epidural anaesthesia, including a new block, results in an almost complete success rate.\(^2\) In an imaging study investigating failed epidurals, incorrect catheter localization accounted for half of the failures, while the remaining patients experienced suboptimal analgesia through a correctly positioned catheter.\(^3\) A flow chart adapted from Kinsella et al. illustrates in exemplary fashion the problems encountered during epidural anaesthesia using the example of a caesarean section, ultimately resulting in a success rate of just 76% (Figure 1).

The present review summarizes technical factors known to influence block success, and gives an overview of the pharmacologic strategies available to optimise epidural anaesthesia and analgesia. For each section, we performed a comprehensive literature search for full published reports in MEDLINE covering manuscripts until October 2011, with reference lists of retrieved articles searched for additional trials or reports. We ranked meta-analyses and randomized controlled trials highest, with other trials and reports resorted to in case no broad evidence base could be discerned.
Technical factors influencing block success

Anatomical catheter location

Epidural catheters may primarily be placed erroneously, or dislodge during the course of treatment. Collier described transforaminal migration of the catheter tip and asymmetric epidural spread as the most important caveats during epidural analgesia.\(^4\) Primary malposition of epidural catheters has been described, among others, in the paravertebral space, in the pleural cavity, and intravascularly. Even when the epidural space is correctly identified, the catheter will not necessarily follow a straight line when being advanced, but may deviate in different directions. The epidural catheter may exit the epidural space via the intervertebral foramen at levels above or below the insertion site (Figure 2). In obstetric patients, failure of epidural analgesia after initial success was observed in 6.8% of a studied cohort.\(^2\) Furthermore, secondary migration of the catheter after successful initial placement can occur. During normal patient movement, epidural catheters may be displaced by centimetres.\(^5\) Hoshi et al investigated 60 patients undergoing lung surgery with a thoracic epidural, with chest radiographs taken pre- and postoperatively. In 24% of the patients, the catheter had migrated more than one vertebral level. In addition to gross body movements, changes in epidural pressure and cerebrospinal fluid oscillations contribute to displacement of epidural catheters.\(^6\) The epidural space is highly compartmentalized and complex structure,\(^7\) which may influence catheter placement. Moreover, midline fat pedicles may form a barrier to spread of local anaesthetics.\(^7\)

Patient position

Patient positioning potentially affects needle placement by changing the conformation of osseous and soft tissues. In addition to the obvious opening of the posterior interlaminar space by spinal flexion, the position of spinal contents is altered. The position of the spinal cord within the spinal canal is not precisely
predictable using parameters such as sex, weight or height. However, the patient assuming a flexed position with the head down will result in anterior motion of the spinal cord at the thoracic level, while the spinal cord and cauda equina are located more posteriorly at the lumbar level. The spinal cord is flexibly attached within the dural sac, and changes position according to gravity when subjects are positioned supine, or laterally.

The sitting position has been described to result in shorter insertion times and a trend toward higher accuracy at first attempt than the lateral position, but at the cost of more vagal reflexes, and with comparable final success rates. When investigating combined spinal-epidural anaesthesia for caesarean section, no differences were reported concerning insertion times, while another study found more technical difficulties in the lateral compared to the sitting position. Lateral positioning increases the distance from skin to epidural space. Finally, the sitting position leads to epidural venous plexus distension, which may increase risk of vascular puncture, especially in parturients.

**Puncture site**

Numerous studies have shown that anaesthetists tend to be inaccurate when determining the precise dermatomal level for neuraxial puncture. Of note, most studies show that there is a clear tendency to puncture more cranially than intended. Suggested approximate vertebral levels of puncture for various types of surgery are given in Table 2.

**Midline vs Paramedian**

Few studies have examined the effect of median versus paramedian needle placement upon block success. In cadavers, using epiduroscopy, paramedian catheters were observed to cause less epidural tenting, and pass cephalad more reliably than median catheters. In patients, Leeda et al. reported
faster catheter insertion times in the paramedian, and higher incidence of paraesthesia in the median group. Adequate local infiltration is a prerequisite for patient comfort during paramedian puncture. Finally, the paramedian approach may be less dependent upon spine flexion. The risk of vascular puncture during epidural catheter placement was not associated with lumbar median or paramedian technique in parturients, while another study suggested more paraesthesia and bloody puncture in non-pregnant adults when the median approach was used.

**Localization of the epidural space**

Inability to correctly insert an epidural catheter in the first place or the number of attempts to catheter placement is not reported in most studies, while differences are likely to exist between the thoracic vs. lumbar approach. For example, Rigg and coworkers were completely unable to localize the thoracic epidural space in 13/447 (2.9%) attempts.

Placement of the epidural catheter in the correct position necessitates tools to identify the epidural space. There is considerable variation in the methods used to confirm epidural needle position. Loss of Resistance (LoR) using saline has become the most widely used method, while LoR to air and the hanging drop technique are less widely used. A meta-analysis by Schier and colleagues in 2009 included 5 RCTs comparing LoR to saline versus air: four in the obstetric population and one in a general patient population, summarizing a total of 4422 patients. No significant difference in any outcome was found, except an absolute 1.5% reduction in postdural puncture headache when using saline vs. air. In the meantime, a clinical trial comparing combined spinal-epidural punctures using air vs. saline found no difference in success rate or adverse events. A recent retrospective study of 929 obstetric epidurals found that when using air for LoR, significantly more attempts were needed as
compared to using saline, with comparable final success rates. Subgroup analyses showed that the use of the “preferred technique” (defined as the technique used by a practitioner >70% of the time) resulted in significantly fewer attempts, a lower incidence of paresthesia, and fewer unintentional dural punctures, irrespective of whether saline or air was used for LoR.

The hanging drop technique depends on negative pressure within the epidural space. Recent experimental evidence suggests that negative pressure is suboptimal in reliably detecting the epidural space, and if at all, the hanging drop technique is prudent only in the sitting position. Of note, identification of the epidural space was reported 2 mm deeper for the hanging drop as compared to LoR, possibly indicating increased risk of dural perforation.

Finally, whatever technique is used, it is of importance to realise that the ligamentum flavum is not continuous in all patients, and the presence of midline gaps may make the loss of resistance to needle advancement and injection of air/saline less perceptible when the median approach is used.

A number of technical aids for epidural anaesthesia have been described, none of them exhibiting sufficient accuracy and practicability to as yet justify the increased effort and cost of their routine use in adults. Ultrasound may serve as an educational tool and enhance the learning curve for epidural anaesthesia, and pre-assessment of lumbar epidural space depth has been shown to correlate well with actual puncture depth in obese parturients. In children, ultrasound allows for identification of neuraxial structures, particularly in neonates. Below an age of 3 months only the vertebral bodies are ossified, enabling detailed visualization of spinal structures. At age 3 months or older, the vertebral column further ossifies, leading to decreased visibility. At approximately age 7 years, visibility of the neuraxial structures, especially the thoracic segments, is significantly reduced and comparable with that of young adults. Despite apparently obvious advantages of ultrasound-guided epidural
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anaesthesia in children, only one randomized controlled trial has been conducted to date. Willschke et al. compared ultrasound to LOR to identify the epidural space. Use of ultrasound led to less bony contact, a shorter time to block success, and decreased supplemental opioid requirements. Recently, visualization of epidural spread of local anaesthetic has been used to predict optimal individual epidural dose. e.g.\(^3\)

**Catheter insertion and fixation**

The catheter should be inserted at least 4 cm into the epidural space,\(^5\) and a recent study reported a higher success rate when slightly more than 5 cm were achieved.\(^3\) Tunnelling the epidural catheter for 5 cm in a cohort of 82 patients was associated with less motion of the catheter, but the percentage of catheters maintaining original position was not statistically different.\(^3\) In more than 200 patients undergoing either thoracic or lumbar epidural anaesthesia, tunnelling led to significantly decreased catheter migration, with a modest clinical net result of 83% of functioning catheters after 3 days, as compared to 67% without tunnelling.\(^3\) Suturing of the epidural catheter was similarly associated with less migration, but at the cost of increased inflammation at the puncture site.\(^3\) Whereas erythema at the puncture site was not associated with bacterial colonization in small-scale studies,\(^3\) one larger study described a positive correlation.\(^3\) In a retrospective observational study involving more than 500 children, tunnelling a caudal epidural catheter reduced risk of bacterial colonization to levels comparable to untunneled lumbar catheters.\(^3\) These results may be related to the fact that tunnelling places the catheter entry point above the diaper in babies and toddlers and may not be easily transferred to an adolescent population undergoing lumbar or thoracic epidural anaesthesia. It seems prudent, however, to consider tunnelling caudal epidural catheters in babies and toddlers. In lumbar and epidural catheters, the advantages are less straightforward and the
necessity to prevent dislodgement (often dependent on type of surgery) needs to be weighed against the increased incidence of erythema at the puncture site, potentially linked to increased risk of bacterial colonization. Catheter fixation devices are available which may significantly reduce migration percentage and reduce rates of analgesic failure.\textsuperscript{40} Unfortunately there are no studies comparing modern dressing devices with tunnelling techniques with respect to migration, analgesic failure, or infection.

\textit{Test dose}

The optimal way to pharmacologically determine position of the epidural catheter has been debated. When administering a test dose, the two main objectives are to detect intrathecal and intravascular catheter placement. The optimal strategy to detect intrathecal catheter placement was long considered to be lidocaine coupled with epinephrine. Specific regimens to detect intravascular catheter position have been advocated for non-pregnant adult patients (fixed epinephrine test dose), parturients (fentanyl test dose), and children (weight-adjusted epinephrine test dose).\textsuperscript{41} It should be kept in mind that a non-significant increase in heart rate (\(<15\%\)) does not guarantee correct position. Furthermore, patients sensitive to intravascular epinephrine (parturients, patients with cardiac or vascular disease) may experience undesired effects in case the test dose is “positive”. However, this risk is most likely outweighed by the deleterious effects of local anaesthetic intoxication should intravascular placement not be detected. Test doses consisting of lidocaine (to detect intrathecal placement) and epinephrine (to detect intravascular placement) are recommended in patients without contraindications to epinephrine.
Material

Problems with the material itself may be responsible for epidural failure. The orifice of the catheter can be in the lateral or anterior epidural space, thereby leaking the local anaesthetic preferably to one side and producing an unilateral block.\textsuperscript{42} In general, multi-orifice catheters are considered superior to single-orifice catheters.\textsuperscript{4,43} Manufacturer’s errors may occur, such as faulty markings on the epidural catheter. This can lead to erroneous depth of catheter placement.\textsuperscript{44} Debridement in the catheter or disconnection may similarly cause epidural failure.\textsuperscript{4} One important preventable cause for obstruction of the epidural infusion system is air lock in the bacterial filter. Depending on the type, as little as 0.3 to 0.7 ml of air is sufficient to cause obstruction.\textsuperscript{45}

Knotting of the catheter inside or outside the body can cause obstruction. Only 13\% of lumbar catheters inserted in a group of 45 men were advanced more than 4 cm without coiling, with coiling occurring at a mean insertion depth of 2.8 cm.\textsuperscript{46} The frequency of knotting catheters is estimated to be 1:20.00-30.000 epidurals.\textsuperscript{47} Based on 18 case reports, Brichant et al. concluded that 87\% of the knots occurred less than 3 cm from the tip of the catheter and that 28\% of the knots were associated with a loop in the catheter.\textsuperscript{47} Removal of a presumably knotted catheter can be attempted after sensitivity has returned to monitor for neurological symptoms during catheter removal. When radicular symptoms or pain occur during removal of a catheter, this should be immediately stopped.\textsuperscript{4} It has been suggested that removal is easiest if position at insertion and removal are similar.\textsuperscript{4} Surgical removal of a broken catheter is not obligatory if the patient remains asymptomatic.\textsuperscript{47}
Pharmacologic optimisation of epidural anaesthesia

Local anaesthetic dose versus volume

The influence of dose, concentration and volume upon spread of epidural anaesthesia and analgesia has been subject of considerable research, and different constellations of volume and concentration have been assessed. In general, the main determinant of epidural action is the local anaesthetic dose, with volume playing a minor role. Thus, quality of epidural analgesia depends on total local anaesthetic dose rather than volume or concentration, either in conventional or patient-controlled epidural analgesia. Although there seems to be a tendency towards more extended sensory block and lower blood pressures with lower concentrations at higher volume \(^48\,49\) and one study even found a higher rate of PONV, \(^50\) most studies did not detect such increased side effects \(^51\,56\).

The situation may be different when local anaesthetic solutions are applied as bolus. There is evidence supporting the role of volume in spread of anaesthesia. For example, the number of dermatomes blocked during labour analgesia was higher in a high-volume bupivacaine group than a low-volume group when the same dose was administered. \(^57\) But again, the evidence is equivocal. Sakura and coworkers found that the spread of lumbar epidural anaesthesia for gynaecological surgery was similar whether 20 ml 1% lidocaine, or 10 ml 2% lidocaine were used. However, the intensity of block was higher in the lido 2% group. \(^58\) If the difference between injected volumes differs by more than 200% for the same concentration, the block will spread further in the high-volume group. \(^59\) For bolus application there is evidence that reducing dose increases probability of differential blockade. In healthy volunteers, dose-dependency of differential blockade was demonstrated at 0.075 and 0.125% bupivacaine. \(^60\) Higher bupivacaine concentrations caused motor block. It should further be kept in mind that differential blockade is a complex phenomenon, in
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part caused by differential conduction block of spinal nerves and roots, and in part by differential central somatosensory integration.\textsuperscript{61} Dose is the primary determinant of epidural anaesthesia, with volume and concentration playing a subordinate role during continuous or patient-controlled epidural anaesthesia (PCEA) application. The effect of volume is more pronounced during bolus application.

Motor block may be more extensive when performing lumbar epidural anaesthesia because of the spatial proximity of motor fibers. This was recently confirmed in audit form.\textsuperscript{34} In labour, low-dose epidural analgesia may be associated with less operative vaginal deliveries.\textsuperscript{62} The use of smaller doses in higher volumes has therefore been advocated for obstetric analgesia.\textsuperscript{63}

Choice of local anaesthetic

The three main long-acting local anaesthetics for epidural anaesthesia and analgesia are bupivacaine, levobupivacaine, and ropivacaine. The prospect of improved differential blockade, and concerns regarding cardiac safety have driven the increased use of the newer L-stereoisomers. The equipotency of these three drugs has been the subject of many clinical studies. For example, equal concentrations and dosing of bupivacaine and ropivacaine (0.125\%, with fentanyl 2mcg/ml) lead to equal efficacy concerning analgesia, but significantly less motor block in the ropivacaine group.\textsuperscript{64} However, comparison of equal doses of, e.g., bupivacaine and ropivacaine is difficult as the difference in potency is approximately 40-50\%.\textsuperscript{65} This has profound consequences because to interpret differential toxicity, this difference in potency needs to be taken into account. If one translates potency difference to the toxic threshold of local anaesthetic causing convulsions in animal models,\textsuperscript{65} near equipotency of bupivacaine and ropivacaine concerning toxicity becomes apparent. The likelihood of successful resuscitation after local anaesthetic intoxication has been described as lower
when bupivacaine is the causative agent based upon receptor binding. However, the therapy advocated to support resuscitation of patients intoxicated with local anaesthetics, intralipid, may be more beneficial in bupivacaine- than in ropivacaine-induced toxicity owing to the lipophilic properties of bupivacaine. There is no evidence to refute bupivacaine in favour of the newer stereoisomers when used for epidural anaesthesia or analgesia in adults. Based upon pharmacologic data, switching local anaesthetics is not likely to improve epidural anaesthesia.

Addition of opiates

The addition of small doses of opiate allows for dose reduction of local anaesthetics while improving quality of analgesia. The vast majority of studies support the use of a combination of local anaesthetic and opioid over either drug alone. In a 1998 meta-analysis, Curatolo et al. showed that epidural fentanyl was a beneficial adjuvant to local anaesthetics administered for surgical analgesia, improving pain therapy with a low incidence of nausea, and rare occurrence of pruritus. The addition of opiates allows for smaller concentrations of local anaesthetic, thereby possibly reducing motor block postoperatively, or during labour. In fact, it has been stated that the entire concept of low-dose local anaesthetics for analgesia is feasible only when opioids are used as adjunct therapeutics. Moreover, recent data suggest that epidural opioids can enhance the quality of suppression of the surgical stress response.

Profound differences exist between hydrophilic opioids (such as morphine) and lipophilic opioids (such as fentanyl and sufentanil). Microdialysis studies demonstrate that epidural morphine has a longer residence time in the epidural space, and results in higher CSF concentrations as compared to sufentanil and fentanyl. This long residence time results in a spinal mechanism...
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of action, and consequently, clinical studies show a substantial dose reduction in morphine when used epidurally instead of intravenously.\textsuperscript{74} Corresponding evidence for lipophilic opioids such as fentanyl and sufentanil, however, is more conflicting. While some studies show a clear benefit of adding epidural fentanyl to bupivacaine,\textsuperscript{75} others suggest that effects of epidural fentanyl are primarily mediated by supraspinal mechanisms after systemic absorption.\textsuperscript{76} In a recent study undertaken in healthy volunteers, differences were observed between continuous and bolus infusion. While continuous infusion resulted in non-segmental analgesia (indicating supraspinal action), bolus injection resulted in segmental analgesia (indicating significant spinal contribution).\textsuperscript{75} Therefore, the elicitation of a spinal analgesic mechanism may depend on sufficient concentrations of fentanyl in the epidural space to allow for diffusion into the CSF, i.e., estimated above 10 mcg/ml, which exceeds current postoperative analgesia regimens.\textsuperscript{77}

Finally, some potential disadvantages of epidural opioid administration should be discussed. First, the safety of opioids in obstetric analgesia has been subject of discussion. Potential disadvantages of epidural opioid application include possible interference with breast-feeding,\textsuperscript{78} but a recent randomized controlled trial found no effect of epidural fentanyl on breastfeeding initiation and duration.\textsuperscript{79} Second, biphasic respiratory depression may occur when hydrophilic opioids are given epidurally. With hydrophilic opioids such as morphine, the first peak corresponds to absorption from the epidural space into the systemic circulation and occurs 30-90 minutes after injection, while a second depression occurs 6-18 hours later as morphine spreads towards the brainstem. In lipophilic opioids, there is only an early depression due to absorption and rostral spread.\textsuperscript{80}
Addition of epinephrine

The addition of adrenaline to epidural solutions causes two desired effects. First, vasoconstriction causes delayed absorption of local anaesthetic into the systemic circulation, with higher effect-site and lower plasma concentrations. Second, adrenaline has specific antinociceptive properties predominantly mediated via alpha-2 adrenoreceptors. Effects of epinephrine with regards to local anaesthetics and opiates are supra-additive. For example, the MLAC of bupivacaine is reduced by 29% in labouring parturients. Adding epinephrine to a low-dose thoracic epidural infusion of ropivacaine and fentanyl improved pain relief and reduced nausea.

Vasoconstriction plays a key role in the supra-additive effect of adrenaline during epidural analgesia. Amide-type local anaesthetics are not metabolized in the epidural space, liver, such that the main determinant for their concentration there is absorption into the systemic circulation and subsequent hepatic metabolism. This absorption is biphasic, with an initial fast peak reflecting the fluid phase and later a slower second peak corresponding to resorption from the lipid compartment at the site of injection. Addition of epinephrine to local anaesthetic solutions slows down the first phase of systemic absorption. The net clinical effect is a more profound block, or sufficient block already at lower LA dose. The same mechanism seems to be valid for opioids.

Further, epidural epinephrine has a specific alpha-2-mediated antinociceptive effect causing decreased presynaptic transmitter release and postsynaptic hyperpolarization within the substantia gelatinosa of the spinal cord dorsal horn. Therefore, the full effect is only observed when the epidural catheter is positioned within the vicinity of the spinal cord, i.e. above L1. Lumbar catheters necessitate higher concentrations of local anaesthetic and opioid, and here, adding epinephrine may increase the possibility of motor block. Studies suggest a concentration of 1.5-2 µg/ml as effective.
Finally, some potential risks of adding epinephrine should briefly be discussed. Potential side effects have been described in obstetrics, the main concern that adrenaline-containing solutions potentially cause longer labor, and decreased uterine blood flow. At doses usually used clinically, spinal cord ischemia seems to be no clinically significant problem.

**Bolus versus continuous dosing**

The advent of PCEA has profoundly changed the administration of postoperative pain treatment. In labour analgesia, a meta-analysis by van der Vyver et al. demonstrated that patients undergoing obstetric PCEA needed less co-analgesic interventions, while requiring less local anaesthetic, and potentially having a decreased likelihood of motor block, albeit without a difference in maternal satisfaction and no effect upon mode of delivery. Using pain scores and cumulative local anaesthetic dose as outcome parameters, conflicting evidence has been put forward to prove or refute background infusions. It is important to keep in mind that PCEA requirement are determined by the site of surgery, and surgery for malignant disease, as well as patient weight and age seem to be the most important predictors. The addition of a continuous infusion to PCEA during labour resulted in reduced total dose of local anaesthetic while providing effective analgesia. A reduction in local anaesthetic dose was found only in demand-only PCEA, but not in the group with background infusion by Vallejo, who found similar outcomes in all groups. Recently, Lim and colleagues showed that demand-only PCEA did result in less LA administered, but also in more breakthrough pain, higher pain scores, and lower maternal satisfaction during labour. More refined techniques such as programmed intermittent epidural bolus (PIEB) combined with PCEA have shown potential for even more accurate analgesia.
Conclusion

Failure of epidural anaesthesia and analgesia occurs in up to 30% in clinical practice. Some technical factors can help to increase primary and secondary success rate. Epidural catheters may be erroneously placed, or may migrate secondarily after initial correct placement due to body movement and oscillations in cerebrospinal fluid. Moreover, catheters may deviate from the midline during insertion. The optimal depth of insertion in adults is approximately 5 cm. The most widely used method with the least side effects for localizing the epidural space is loss of resistance to saline. None of the additional technical tools available has sufficient accuracy and predictability to justify routine use, with the exception of a growing evidence-base for ultrasound in obese patients and infants. The optimal test dose should combine lidocaine (to detect intrathecal placement) and epinephrine (to detect intravascular placement) in the absence of contraindications to epinephrine. In the case of catheter knotting, direct retrieval of the catheter should be attempted once anaesthesia has worn off, while surgical intervention is rarely indicated. The choice of long-acting local anaesthetic seems to be less important clinically. Dose is the primary determinant of continuous epidural anaesthesia, with volume and concentration playing a subordinate role. Addition of opiates may substantially increase the effectiveness of epidural analgesia. Epinephrine strengthens analgesia by delaying resorption of local anaesthetic from the epidural space, and by direct antinociceptive action at the spinal cord. The method most supported by literature for postoperative analgesia is patient controlled epidural analgesia with background infusion.

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### Table 1 – Definitions and rates of failed epidural anaesthesia or analgesia

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>Type of epidural</th>
<th>Definition</th>
<th>Failure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstetric</td>
<td>Lumbar</td>
<td>Any reason for intervention</td>
<td>550/4240 (13.1%)</td>
</tr>
<tr>
<td>Eappen et al.</td>
<td>IJOA 1998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General surgery</td>
<td>RAPM 1999</td>
<td>Technical defects / Insufficient block</td>
<td>N=2140 thoracic (32%) lumbar (27%)</td>
</tr>
<tr>
<td>McCleod et al.</td>
<td>Anaesthesia 2001</td>
<td>Technical defects / Insufficient block</td>
<td>83/640 (13.0%)</td>
</tr>
<tr>
<td>Gastrointestinal surgery</td>
<td>Rigg et al. 2002</td>
<td>Failed insertion / Removed early</td>
<td>203/431 (47.1%)</td>
</tr>
<tr>
<td>Esophagectomy</td>
<td>RAPM 2003</td>
<td>Catheter dislodgement</td>
<td>8/46 (14.2%)</td>
</tr>
<tr>
<td>Obstetric</td>
<td>IJOA 2004</td>
<td>Inadequate analgesia, spinal tap</td>
<td>1099/7849 (14%)</td>
</tr>
<tr>
<td>Abdominal oncology</td>
<td>Motamed et al. 2006</td>
<td>Failed block, premature removal of catheter</td>
<td>31/125 (24.8%)</td>
</tr>
<tr>
<td>Whipple</td>
<td>Pratt et al. 2008</td>
<td>Failed block, premature catheter removal</td>
<td>49/158 (31.0%)</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Study</th>
<th>Type of surgery</th>
<th>Level of neuraxial block</th>
<th>Cause of failure</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinsella et al. 2008</td>
<td>C-section Lumbar</td>
<td>Inadequate anesthesia</td>
<td>302/1286 (23.5%)</td>
<td></td>
</tr>
<tr>
<td>Königsrainer 2009</td>
<td>Major surgery</td>
<td>Insufficient analgesia, motor block, catheter dislodgement</td>
<td>124/300 (41.4%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 - Landmarks for epidural anesthesia and analgesia**

**Desired dermatome level of neuraxial block**

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>Upper dermatomal block level</th>
<th>Anatomic landmark</th>
<th>Optimal insertion point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus, lung</td>
<td>T1</td>
<td>Below clavicle</td>
<td>T6-7</td>
</tr>
<tr>
<td>Upper abdomen</td>
<td>T1</td>
<td>Below clavicle</td>
<td>T9-10</td>
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<tr>
<td>Lower abdomen</td>
<td>T6</td>
<td>Distal sternum</td>
<td>T9-10</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>T4</td>
<td>Nipples</td>
<td>L4-5</td>
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<tr>
<td>Lower extremity</td>
<td>L1-2</td>
<td>Inguinal crease</td>
<td>L4-5</td>
</tr>
</tbody>
</table>
Regional anesthesia and risk of nerve damage: 
influence of pre-existing neuropathy
Chapter 3.1

*Effects of needle trauma and intraneural injection in vivo*

Based on

*Kirchmair L, Loescher W, Voelckel W, Lirk P*

Short-term neurophysiologic and morphologic effects of experimental needle trauma and intraneural injection.

*Br J Anaesth 2014; (in revision)*
**Introduction**

Peripheral nerve injury is a rare but severe complication after peripheral regional anaesthesia.\(^1\) Several mechanisms are thought to contribute to transient or even permanent neurologic deficit, such as local anaesthetic toxicity, mechanical nerve damage, and ischemia.\(^2\) In the perioperative period, the contribution of additional patient risk factors such as a pre-existing neuropathy is controversially discussed.\(^3\) Substantial evidence supports the notion that injury to a nerve because of needle trauma and subsequent intraneural injection is harmful.\(^2\) On the other hand, some recent publications have suggested that intraneural injection, if performed outside of individual nerve fascicles, is not invariably deleterious \(^4\) and may even be safe.\(^5\)\(^6\)

Previous investigations have been carried out, using neurohistopathology and neurobehavioral testing as endpoints.\(^7\)\(^8\) No model has been described which would allow for the detailed measurement of electrophysiologic variables following nerve injury. We sought to describe a large animal model of peripheral nerve blockade using the same equipment and techniques as in clinical practice, to investigate functional effects of intraneural needle placement and fluid injection.

The aim of this study was to test the hypothesis that intraneural fluid injection, but not intraneural needle placement, would result in functional nerve damage as evidenced by a decrease in compound motor action potential (cMAP) amplitude.

**Methods**

**Animals**

The experimental study protocol was approved by the Animal Care and Use Committee of the Austrian Federal Ministry of Science (GZ BMBWK-66.011/0065-BrGT/2006). We tested 15 healthy research pigs, aged 12 to 16
Needle trauma and intraneural injection

weeks and weighing 22 ± 2 kgs. Animals were fasted overnight with free access to water, and premedicated with azaperone 4 mg kg\(^{-1}\) i.m. and atropine 0.1 mg kg\(^{-1}\) i.m.. After a bolus of ketamine 20 mg kg\(^{-1}\) i.m., peripheral venous access was established. Anaesthesia was induced and maintained using propofol 2-3 mg kg\(^{-1}\), followed by 1.5 mg kg\(^{-1}\) h\(^{-1}\). The trachea was intubated in the spontaneously breathing supine animal. After intubation, animals were given piritramide 15 mg i.v., repeated as necessary. Mechanical ventilation was initiated with an inspiratory oxygen fraction of 35%, positive end-expiratory pressure of 5 cm H\(_2\)O, a respiratory rate of 20 breaths per minute, with tidal volume adjusted to maintain normocapnia. In parallel, lactated ringer’s solution 6 ml kg\(^{-1}\) h\(^{-1}\) and gelatine solution 3% 4 ml kg\(^{-1}\) h\(^{-1}\) were infused. Monitoring included a standard two-lead electrocardiograph, pulse oximetry, and invasive blood pressure monitoring. All animals were euthanized by means of propofol 3 mg kg\(^{-1}\), piritramid 30 mg, and potassium chloride 20 ml to induce cardiac arrest.

Experimental procedures

Animals were randomly assigned to one of four groups before baseline measurements. In Group A (n=5), the sciatic nerve was pierced under ultrasound guidance (needle trauma) and the needle was immediately retracted. In Group B (n=6), 2.5 ml of saline were injected into the sciatic nerve, while in Group C (n=6), 5 ml of saline were injected intraneurally. Using ultrasound, intraneural injections were performed extrafascicularly. In control Group D (n=5), 5 ml of saline were injected around the sciatic nerve to simulate a peripheral nerve block. We obtained electrophysiological recordings at baseline, immediately following nerve injury with or without fluid injection, and after 30, 60, 120, and 180 minutes. The diameter of the sciatic nerve was recorded parallel to the electrophysiologic measurements to calculate its cross-sectional area.
Sciatic nerve interventions

Sciatic nerve interventions were performed using both ultrasound guidance and nerve stimulation. The sciatic nerve was visualized in a short-axis view using a portable ultrasound device dedicated to veterinary use (SonoSite Titan, SonoSite Inc., Bothell, WA) and its diameter (length, width) was recorded. The cross-sectional area of the latter was assumed elliptic and therefore calculated with the formula $\pi \times \frac{1}{4} \times \text{length (mm)} \times \text{width (mm)}$. A needle (UniPlex NanoLine, 20 gauge, facet tip with 45° bevel, 150mm, Pajunk, Geisingen, Germany) was introduced in plane with the ultrasound probe, and advanced to the nerve. Correct needle position was confirmed by means of a standard nerve stimulator (Stimuplex HNS 11, B Braun, Melsungen, Germany) set to deliver a stimulus of 0.3 mA at a frequency of 1 Hz to elicit plantar flexion. Subsequently, interventions were carried out as described above. After all interventions a surgical suture was introduced through the needle to mark the site of intervention for later examination after excision.

Fluid injection

To apply fluid with comparable hydrostatic pressure, we employed a perfusion pump (Perfusor fm, B Braun, Melsungen, Germany) connected to a pressure transducer via an arterial tubing line (Monitoring Set, Medex Medical Inc., Haslingden, Rossendale, Great Britain). We continuously measured pressures within the perfusion syringe, within the fluid line, and proximal to the needle (given in mmHg), and determined average and peak pressures. Volume application was performed using the bolus function of the perfusion pump preset to deliver 10 ml min$^{-1}$ (0.167 ml sec$^{-1}$), resulting in 15 and 30 seconds to inject 2.5 and 5 ml, respectively. Real-time visualization was used in order to prove intraneural injections by nerve expansion. Baseline injection pressure (syringe
and tubing) without tissue resistance was obtained in a separate experiment (n=10).

Electrophysiology

*Compound motor evoked potentials (cMAP):* The sciatic nerve was stimulated at the gluteal level using a stimulating needle-electrode (Medtronic A/S, Skovluunde, Denmark) which was placed under ultrasound guidance to avoid needle-nerve contact. A surface electrode (TECA Disposable Surface Electrodes, Oxford Instruments, Medical Systems Division, Pleasantville, NY, USA) served as anode and was placed in the groin area opposite to the stimulating electrode. The cMAPs were recorded by means of two surface electrodes placed over the gastrocnemius muscle and its insertion point, respectively. A standard ECG-electrode (Skintact, Innsbruck, Austria) was used as reference electrode. To determine optimal intensity of stimulating current, we used the “inching” protocol of the Nicolet Viking IV device (Nicolet Biomedical, Madison, WI, USA) at the baseline of each experiment. In this protocol, the sciatic nerve was stimulated with a square wave pulse of 0.2 ms duration, and increasing stimulating current until the amplitude of cMAPs over the gastrocnemius muscle did not increase further. This current was increased by a additional 20-50% to assure supra-maximal stimulation intensities throughout the experiment. Amplitude (negative peak given in mV, reflecting axonal damage) and distal motor latency (given in ms, and reflecting myelin damage) of cMAPs were recorded as the average of five single measurements.

Macroscopic examination

After animals had been euthanized, sciatic nerves and adjacent tissues were excised, taking care not to distort anatomy. The sciatic nerves were exposed
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beyond the previously marked site of intervention and their macroscopic appearance was documented by means of digital imaging.

*Power Calculation and Statistics*

The sample size used would permit a detection of a difference in primary outcome (cMAP amplitude) characterized by difference in means of 40%, assuming a common standard deviation of 20%, a Power of 80%, and assuming statistical significance at $P < 0.05$.

Analysis of Variance with post hoc correction (LSD) was used to compare cMAPs (amplitudes, latencies) and sciatic nerve cross sectional areas. Normal distribution of sample values was checked for using the Kolmogorov-Smirnov Test. SPSS for Windows v11.0 (Chicago, IL) was used for all analyses, and differences were considered highly significant at $P < 0.001$.

*Results*

*Injection pressures*

Baseline injection pressure (syringe and tubing) without tissue resistance was 63.6 ± 3 mmHg. Maximal pressures were not different between the four experimental groups (Table 1). In two instances, maximal pressures of up to 320 mmHg were reached in the control group, and sonographic control of needle position showed a fascial layer obstructing fluid outflow.

*Sonography*

The cross-sectional area of the sciatic nerve as determined by ultrasound did not change after nerve trauma and in the control group. After injection of 2.5 ml or 5 ml of saline, significant changes in nerve diameter were discernible (Figures 2a, 2b). One sciatic nerve injected with 5 ml saline exhibited rupture of the epineurium observed by means of real-time ultrasound imaging.
accompanied by a sudden decrease of injection pressure. In some other nerves, we observed circumstantial evidence of the same event, with a sudden drop in nerve cross-sectional diameter after presumed rupture of the nerve epineurium.

**Electrophysiology**

We determined both amplitude and latency of cMAPs. Amplitudes and latencies were normalized to baseline measurements before needle trauma or injection. Absolute and normalized amplitudes as well as latencies were distributed normally. Decreases in amplitude were significant after needle trauma (Group A) and injection of 2.5 ml intraneurally (Group B), and highly significant after injection of 5 ml (Group C) (Figure 1). Amplitude did not change in Group D (controls). Normalized amplitudes are summarized in table 2. Latency of nerve conduction did not change over time in any group.

**Macroscopy**

The harvested nerves showed apparent signs of injury (haematoma, swelling) after needle trauma (group A) (Figure 3) and injection of 2.5 ml and 5 ml (groups B and C), respectively. In the control group (group D) the nerves showed no signs of injury.

**Discussion**

The aim of this study was to test the hypothesis that intraneural fluid injection, but not intraneural needle placement, would result in functional nerve damage as evidenced by a decrease in cMAP amplitude. We report that cMAP amplitude as a marker of axonal injury decreased significantly following both intraneural needle placement and intraneural fluid injection.
Injection pressure

Measurement of maximal injection pressures showed that values were not different among the experimental groups and below the threshold of 15 psi (≈ 776 mmHg), suggesting extrafascicular injection.\textsuperscript{11} This is in line with recent evidence in human cadavers showing high injection pressures during injection into brachial plexus nerve roots exceeding by far any pressures encountered during the present investigation.\textsuperscript{9} The fact that we observed pathological changes even after extrafascicular injection cannot be extrapolated directly to the clinical situation, but would lead us to question the purported safety of intraneural extrafascicular injection.\textsuperscript{5}

Sonography

In our study, ultrasound was used to perform intentional needle trauma and fluid injections into the sciatic nerve. Real-time imaging was applied to assure intraneural injection by monitoring nerve swelling as a reliable sign of true intraneural injection, be it subepineural or subperineural.\textsuperscript{10} Our findings confirm and widen previous reports by Chan et al.\textsuperscript{11} in as much as we visualized the injection of fluid in real-time using ultrasound. We found no changes in nerve diameter after needle trauma or extraneural injection whereas following intraneural injection, the diameter of affected nerves was increased over several hours. This provides circumstantial evidence of a fluid depot remaining within the nerve, potentially leading to compression of nerve fascicles. We demonstrate a fluid reservoir within nerves after injection, which persisted over the entire experimental timescale, i.e. 3 hours (Figure 2b). Mechanical compression is a well-established cause of neuronal damage and a valid method of temporary neurolysis, e.g. in trigeminal neuralgia.\textsuperscript{12} In contrast, the extraneural fluid reservoir injected in control animals was significantly decreased in volume after three hours.
Whereas needle trauma typically led to formation of small haematoma without further macroscopic evidence of nerve damage, we found no macroscopic evidence of injury in our control group. In analogy, sonographic appearance of nerves following needle trauma alone revealed no abnormal findings. We note that in a recent study, paraesthesia during needle placement as potential indicator of needle-nerve contact or even trauma was associated with new-onset neurological symptoms. It should be noted that in the present study, nerve diameter was not used as surrogate marker of nerve damage, rather it was used to verify correct localization of injectate. We quantified nerve damage on basis of electrophysiological parameters.

**Electrophysiology**

Using electrophysiological monitoring, we demonstrate a decrease in amplitude of cMAP after needle trauma and intraneural injection of either 2.5 or 5 ml saline, reflecting neuronal damage by direct axonal injury, and, potentially, mechanical compression of neuronal structures by fluid reservoirs. In detail, cMAP amplitude decreased by about 30% of baseline values after needle trauma, and by up to 70% after intraneural injection. Based upon the injection pressures observed in our trial, and real-time imaging using ultrasound, we surmise that our injections were intraneural, but extrafascicular. The present results lead us to conclude that any impact on the integrity of a nerve leads to functional impairment. The latter appears less pronounced after needle trauma alone as compared to additional intraneural injection. However, the observed reductions in cMAP amplitudes do not allow us to predict the degree of long term injury.

**Intraneural injection in clinical practice**

The question how often nerve injury occurs in clinical practice has been controversially discussed, and based upon study design and definition of
endpoint, the reported incidences range up to 80%. While some investigators have shown that even paraesthesia is a risk factor for neuropathy following regional anaesthesia, some smaller studies found no neurological sequelae of intraneural injection. However, some of the latter studies performed injection within the connective tissue sheath surrounding tibial and common peroneal nerve, with no evidence of structural harm to the constituent nerves, such that these results may not fit the classical description of “intraneural” injection. Our findings support the hypothesis that nerve damage by needle trauma or intraneural fluid injection leads, at least temporarily, to impairment of nerve function, even if we cannot exclude the possibility of long-term recovery of nerve function in our model.

Methodology and Limitations

The main methodological difference between our study and others was that we used online recorded electrophysiologic measurements as surrogate markers of nerve damage. Ultrasound guidance was used throughout all interventions to preserve the complex physiological environment of peripheral nerves and monitor intraneural injections. This design is in contrast to “open” animal models of nerve damage used in some other studies. The injection speed chosen for the present investigations (10 ml min⁻¹) can be considered low when compared with clinical practice. Therefore, if anything, our study design underestimated the pressures generated during intraneural injection, and the damage caused by pressure.

Finally, some limitations of the present study should be briefly discussed. First, since our experiment was conceptualized as a large animal study, we were limited in the observation of long-term effects of nerve injury. It would have been of interest to observe the time-course of nerve trauma over a longer period, since a final determination of neurological damage is only
Needle trauma and intraneural injection

considered feasible after 10-14 days. An additional method offering valuable information is neurohistopathology after nerve trauma. Other authors have described inflammatory changes on sciatic nerves even after regular nerve stimulation, noting lymphocytes and granulocytes six hours after nerve block. Also, we note that our diagnosis of intraneural and extrafascicular injection was based on surrogate parameters such as injection pressure and spread of injectate within the nerve. In future studies, neurohistopathology should be integrated to confirm structural damage and site of injection.

Ethical considerations

To minimize the number of test animals, our experiments were conducted as addendum to a study investigating the inhalative application of N-chlortaurin in the porcine model. We sought to minimize animal distress by conducting all invasive procedures such as electrophysiology and nerve block under general anaesthesia. Experimental procedures, data collection and presentation were in accordance to the ARRIVE guidelines.

Conclusion

Attesting to the fact that our model was not designed to detect long-term recovery of nerve function, we report signs of acute functional neuronal damage after needle trauma and intraneural extrafascicular injection.

References


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Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Injection Pressure</th>
<th>Min</th>
<th>Max</th>
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<tr>
<td>Group B</td>
<td>6</td>
<td>164.5 ± 77.2</td>
<td>100</td>
<td>300</td>
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<tr>
<td>Group C</td>
<td>6</td>
<td>112.8 ± 23.6</td>
<td>90</td>
<td>145</td>
</tr>
<tr>
<td>Group D</td>
<td>5</td>
<td>203.2 ± 103.6</td>
<td>100</td>
<td>320</td>
</tr>
</tbody>
</table>

Injection pressures for Group B (injection of 2.5 mL intraneurally), Group C (injection of 5 mL intraneurally), and Group D (injection of 5 mL extraneurally), using a predefined injection speed of 10 ml min\(^{-1}\). No injection was performed in Group A (needle trauma). Injection pressures given as mean ± standard deviation (mmHg), supplemented by minimal and maximal values for each group.

Table 2

<table>
<thead>
<tr>
<th>Group (control)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>intervention</td>
<td>0.9 (0.2)</td>
<td>0.7 (0.3)</td>
<td>0.6 (0.3)</td>
<td>1 (0.2)</td>
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<tr>
<td>30min</td>
<td>0.8 (0.1)</td>
<td>0.7 (0.4)</td>
<td>0.7 (0.3)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>60min</td>
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<td>0.6 (0.3)</td>
<td>0.5 (0.2)</td>
<td>1 (0.2)</td>
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<tr>
<td>120min</td>
<td>0.6 (0.2)</td>
<td>0.5 (0.3)</td>
<td>0.5 (0.1)</td>
<td>0.9 (0.1)</td>
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<tr>
<td>180min</td>
<td>0.6 (0.2)</td>
<td>0.4 (0.3)</td>
<td>0.3 (0.1)</td>
<td>0.1 (0.1)</td>
</tr>
</tbody>
</table>

Normalized amplitudes (mean, SD in parentheses) of cMAPs at baseline (bl), immediately after intervention (i), and follow-up measurements at 30, 60, 120, and 180 minutes. Group A (needle trauma), group B (injection of 2.5 ml intraneurally), group C (injection of 5 ml intraneurally), group D (injection of 5 ml extraneurally).
Needle trauma and intraneural injection

Figure 1
Compound motor evoked potentials (cMAPs) after intraneural injection of 5 ml saline. Recordings were obtained at baseline (top), immediately following intraneural injection, and after 30, 60, 120, and 180 minutes. The corresponding amplitudes are given in mV.

Figure 2a
Sciatic nerve in the gluteal region before intervention. +: sciatic nerve (short axis view)

Figure 2b
Sciatic nerve in the gluteal region immediately after intraneural injection of 5 ml saline. +: sciatic nerve (short axis view), x: fluid depot

Figure 3
Sciatic nerve after needle-trauma (excised). +: sciatic nerve. x: formation of small haematoma at puncture site.
Figure 1
Figure 3
Regional anesthesia in patients with pre-existing neuropathy: current knowledge

Based on
Lirk P, Birmingham B, Hogan QH
Regional anesthesia in patients with pre-existing neuropathy
Int Anesthesiol Clin 2011; 49: 144-65
Introduction

Regional anesthetic (RA) techniques are increasingly advocated in the perioperative management of ambulatory surgery.\(^1\) Promising evidence shows that RA (i) leads to a reduction in the amount of opioid consumption, thus decreasing post-operative nausea / vomiting (PONV), and (ii) improves pain relief. Considering that pain and PONV are the top two reasons for prolonged hospitalization\(^2\) or re-hospitalization\(^3\) after ambulatory surgery, the use of regional techniques is likely to continue to rise for the foreseeable future. Moreover, recent evidence has widened the spectrum of use of RA blocks into the post-discharge period.\(^4\) As the indications for regional anesthesia and analgesia procedures in ambulatory surgery increase,\(^5\) and the contemporary boundaries of patient selection are pushed further outward,\(^6\) an increasing number of ambulatory surgery patients will present for RA with substantial co-morbidities, including neurologic disease.

Neuropathies are a heterogeneous group of neurological conditions secondary to genetic, inflammatory, metabolic, or mechanical injury of nervous tissue. Their relevance in the perioperative management for ambulatory surgery lies, to a large extent, in the deliberation of whether to include a regional technique in the anesthesia plan. This becomes especially important in the case of patients suffering from long-standing diabetes mellitus (DM), whose occurrence in daily practice is projected to rise steadily, and who are often affected by diabetic peripheral neuropathy (DPN).\(^7\) Intuitively, it would seem logical that preexisting metabolic or structural neural defects increase the risk of nerve damage after RA. For decades, anesthesiologists have cautioned against RA in patients with preexisting neural disease.\(^8\) The widely accepted notion by Selander et al. that every nerve block entails a certain degree of (reversible) neuronal injury would lend support to this theory.\(^9\) However, current literature does not give definitive answers.
The aim of this review is to review the pathogenesis and incidence of nerve injury, to discuss implications of preexisting neurological disease upon the occurrence of nerve injury in the context of RA, and to describe strategies that may minimize the risk of iatrogenic aggravation of a preexisting neuropathy.

Nerve Injury during RA

Nerves are exposed to multiple risks during RA from the converging effects of local anesthetic (LA) pharmacology, technical aspects of nerve block, patient factors, and surgical factors (e.g., tourniquet). Only a minority of nerve injuries are caused by direct solitary mechanical trauma sufficient to damage a nerve permanently (e.g. intraneural injection).

Often, the toxicity of LAs and adjuvants is directly implicated in nerve injury, and a large body of literature supports the notion that LAs are directly neurotoxic. In cell cultures, dose- and time-dependent elicitation of cell death from LAs is observed, while in animal models, signs of nerve damage are evident even at clinically relevant doses. In patients, application of LA during nerve block results in very high concentrations of these drugs in target tissues, resulting in disruption of calcium signaling, triggering a cascade of events leading to programmed cell death. A plethora of contributing events has been investigated, some providing a potential link to preexisting neuropathy. For example, lidocaine selectively activates the pro-apoptotic p38 mitogen activated protein kinase in primary sensory neurons, causing neurotoxic effects. The same pro-apoptotic pathway is activated in neurons during DPN, while its inhibition diminishes the functional impact of neuropathy. It is thus conceivable that lidocaine is more toxic in nerves that are already prone to apoptosis owing to activation of the same deleterious pathway by both neuropathy and LA. This concept would, on a molecular level, be in analogy with the “double-crush hypothesis”, which states that preexisting injury of a
neuron leads to a supra-additive effect of a second injury harming the same nerve.\textsuperscript{18}

LAs can also damage nerves by interrupting blood flow, owing to vasoconstrictive properties of anesthetics such as lidocaine and bupivacaine.\textsuperscript{14} Direct mechanical damage by laceration of the perineurium leads to a disruption of the blood-nerve barrier and nerve edema.\textsuperscript{14} However, it is thought that permanent nerve damage is unlikely unless needle penetration is followed by intraneural injection of LA. Structural examinations show that injection of saline produces no long-lasting pathologic changes.\textsuperscript{19} Recently, it has been suggested that intraneural injection may not be necessarily harmful,\textsuperscript{20} and it would seem that nerve anatomy is supportive of this notion. Peripheral nerves are composed of nerve fibers as well as abundant amounts of connective tissue, especially at regions where nerves are exposed to mechanical and shear stress, such as across joints, so, a needle may pierce a nerve without damaging neural structures.\textsuperscript{14, 21} Nevertheless, it must be recognized that for commonly used LAs, toxicity is most likely influenced by the time and concentration of exposure of the peripheral nerve to the LA.\textsuperscript{14} Intraneural injection prolongs the exposure and increases the concentration of LA, and may reduce intraneural blood flow. Because of the potential risk of injuring nerve fibers upon intraneural needle placement and subsequent injection of LA, we believe the safest option is to avoid intraneural needle placement during anesthetic injection procedures. This concern particularly applies to neuropathic nerves.

Nerve injury can also be a consequence of general anesthesia (GA),\textsuperscript{22, 23} sedation,\textsuperscript{24} or surgery \textit{per se}.\textsuperscript{25} For example, after shoulder surgery, interscalene block is a less frequent cause of postoperative neuropathy than surgical factors.\textsuperscript{25} The term “coincident injury” describes an injury occurring during nerve block which is unrelated or only indirectly related to RA.\textsuperscript{26} One example is neuropathy caused by tourniquet inflation during total knee arthroplasty.\textsuperscript{27} RA is perhaps
more conspicuous than surgical events as a risk factor for nerve injury in the perception of patients and surgical colleagues, such that any injury evident after surgery during which RA was used is often, perhaps incorrectly, first attributed to the nerve block technique. This has the potential to mask the true cause of neurological injury and to delay appropriate therapy. At times, surgical risk factors may overlap with anesthesia-related factors to elicit nerve irritation or injury. For example, the association of toxicity (spinal lidocaine) and surgical positioning (lithotomy) in the pathogenesis of transient neurological syndrome has been demonstrated.

**Incidence of Nerve Injury after RA**

The incidence of nerve injury after RA has been reported with widely diverging results. RA procedures feature prominently in closed claims databases, with most complications, and all fatalities, occurring after neuraxial blocks, and a smaller number of injuries being reported after peripheral nerve blocks. Data of outcome studies shows that the risk for neuropathy following epidural and spinal anesthesia is 3.78 : 10,000 and 2.19 : 10,000, respectively. After peripheral nerve block, the incidence of transient neuropathy is less than 3 : 100, whereas permanent nerve damage is very rare. Recently, Watts *et al.* investigated outcomes after 1065 peripheral nerve blocks, and found one transient and one permanent case of nerve injury, amounting to an overall incidence of block-related nerve injuries of 0.22%. The Australasian Regional Anesthesia Collaboration investigated 7156 blocks in 6069 patients, and found thirty patients with potential nerve damage (0.5%), but at analysis, only 3 had neuropathy attributable to RA, giving an incidence of 0.04%. Another investigation reported two cases of neuropathy following continuous popliteal sciatic nerve block in 400 patients, resulting in a risk of 0.5%.
Evidence for elevated risk in patients with preexisting neurological disease

For several decades, there has been a growing consensus that preexisting nerve injury increases the risk of neuropathy in patients undergoing surgery. Originating from the first recommendations to avoid neuraxial anesthesia in patients with preexisting neuropathy,\textsuperscript{35} it was later hypothesized that preexisting neuropathies may predispose peripheral and central nerves to subsequent injury during RA.\textsuperscript{18} Today, a more nuanced view has been adopted. While in some preexisting conditions, we are now more ready to administer RA and analgesia after a careful weighing of risks and benefits (e.g., obstetric epidural analgesia in patients suffering from multiple sclerosis), other neuropathies such as diabetic neuropathy are increasingly recognized as serious risk factors for postoperative neuropathy that may warrant a change of current practice.

In many patients, preexisting neurologic disease may represent an important risk factor, most notably in diabetic peripheral neuropathy, multiple sclerosis, Guillain-Barre syndrome and Post-polio syndrome.

Specific disease patterns

\textit{Diabetic peripheral neuropathy}

Diabetes mellitus is a disease increasing in prevalence in most high-income countries,\textsuperscript{7} leading in turn to an increasing prevalence of DPN. It is estimated that about 10\% of diabetic patients present with DPN at the time of diagnosis, while this number rises to more than 50\% of diabetic patients 5 years later,\textsuperscript{36} probably even faster in poorly controlled DM type 1.\textsuperscript{37} Diabetic neuropathy is the most common neuropathy worldwide, and can be subdivided into distal symmetric, autonomic, and focal/multifocal forms.\textsuperscript{38} Most patients suffer from a chronic distal symmetric nerve injury involving both sensory and motor fibers. Symptoms include hypesthesia and dysesthesia. Due to the
development of symptoms in long fibers of the lower extremity, and the gradual spread to shorter axons, this variant has been designated length-dependent diabetic polyneuropathy.\(^{38}\) Whereas the traditional distal symmetric neuropathy is predominantly driven by metabolic factors, focal neuropathies seem to be caused by a combination of ischemic and inflammatory processes.\(^{38}\) Progression of DPN is difficult to predict in the individual patient, but poor glycemic control seems to be the main risk factor.\(^{39}\) Recently, genetic polymorphisms in the aldose reductase gene \textit{AKR1B1} have been reported to influence the severity and progression of the disease.\(^{40}\) Current evidence suggests that once structural damage has occurred, damage to end organs is irreversible.\(^{38}\)\(^{39}\) This is relevant to daily anaesthetic practice because on the one hand, cardiovascular and renal complications of this disease lead to many surgical procedures for which RA may be highly suitable. On the other hand, limited epidemiological\(^ {41}\) and experimental\(^ {42}\) evidence suggests an increased risk of local anaesthetic-induced neurotoxicity in this growing patient population. It should be kept in mind, however, that up to 30% of neuropathies in diabetics may be unrelated to diabetes.\(^ {43}\)

Concern regarding the performance of RA in diabetic patients has been promoted by epidemiological, experimental, and anecdotal observations. Retrospective data compiled by Hebl et al. indicate that preexisting DPN may be a risk factor for nerve injury after neuraxial anesthesia.\(^ {41}\) Even though direct extrapolation of the results from this study is difficult owing to the small number of complications observed (n=2), it is worth noting that these iatrogenic injuries occurred in diabetic patients using full doses of 0.5 or 0.75% of neuraxial bupivacaine.\(^ {41}\) The risk of nerve damage was described as 10-fold higher in DPN\(^ {41}\) than the estimated 0.04% reported for the general patient population.\(^ {31}\)

Experimental evidence supports increased toxicity of LA in diabetic test animals.\(^ {42}\)\(^ {44}\) In a landmark study, Kalichman and Calcutt found that 2% lidocaine
used for sciatic nerve block in rats produced moderate nerve injury in healthy animals, but induced severe degeneration in diabetic animals. Recently, Kroin et al. showed that lidocaine 1% was safe in a comparable setting for peripheral nerve blockade, whereas there was slight evidence of nerve injury when lidocaine was used with additives (clonidine, epinephrine), or ropivacaine 0.5% was used. Anecdotal evidence lists several patients who experienced worsening of neurologic function after peripheral or neuraxial anesthesia. The extent of preexisting DPN in these patients ranged from subclinical to severe, and included the combination of both diabetic and alcoholic neuropathies.

Other concerns in diabetic patients pertain to pharmacology of LA on diabetic nerves, and the extent to which diabetic nerves are amenable to peripheral nerve stimulation. While Kalichman and Calcutt did not find prolonged block in test animals, the recent study by Kroin et al. showed prolongation of block duration in diabetic animals as compared to healthy controls. It seems, therefore, that diabetic nerves are more sensitive to LA. In fact, regional blockade may be paradoxically efficient in diabetic nerves owing to three reasons. First, nerves may be more sensitive to the LA itself, second, the sensory area of a nerve may be partly anesthetized by neuropathy itself, and third, microangiopathy may contribute to delayed absorption of LA. The possibility of altered responsiveness of diabetic nerves to electric stimulation was first raised in case reports demonstrating difficult stimulation of peripheral nerves in diabetic patients. It would seem logical that a nerve that does not transmit usual impulses properly might be relatively resistant to nerve stimulation, itself a variable undertaking. We have reported that the risk of intraneural needle placement is increased in diabetic dogs compared to controls when using a nerve stimulator to guide needle placement. A more recent report combining ultrasound and nerve stimulation showed that for successful supraclavicular block, higher thresholds are necessary in diabetic versus non-
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diabetic patients. DPN may thus complicate nerve stimulation, possibly resulting in increased incidence of unsuccessful nerve stimulation, or even intraneural needle placement, which may predispose to nerve injury. It may be appropriate in patients with severe DPN to adapt the stimulating patterns to longer pulses, as has been suggested in diabetic/ischemic neuropathy (i.e., from 0.1 msec to 1 msec), or employ ultrasound imaging to improve the accuracy of needle placement.

Whether for central or peripheral blockade, the degree of risk from the use of RA in diabetic patients cannot be exactly defined on the basis of current knowledge. Nonetheless, nerves in DPN are probably more sensitive to LA effects, including toxicity; we recommend reduction of the LA concentration for neural blockade in such patients. Retro-fitting of data from animal experiments using US FDA guidelines allows for estimation of safest doses in patients on the basis of current knowledge (Table 1). Dose reduction may be aided by the employment of ultrasound-guided RA in peripheral nerve blocks. The addition of adjuvants without a corresponding decrease in LA concentration is, in general, not recommended. Diabetic nerves may already be at risk of neuronal ischemia and infarction owing to changes in endoneurial small vessels. Since lidocaine is vasoconstrictive itself, we recommend avoiding epinephrine during nerve blocks in DPN patients.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic demyelinating neuroinflammatory disease. Its etiology is still debated, but a crucial issue is the interplay between polygenetic risk factors (HLA haplotypes) and environmental factors (viral infections, vitamin D deficiency, smoking). Manifestations typically appear in early adulthood, and as in many autoimmune diseases, women are predominantly affected. MS is the most common debilitating disease in young adults.
Diagnosis is primarily based upon the triad of neurologic symptoms, positive findings on magnetic resonance imaging, and the presence of oligoclonal bands on cerebrospinal fluid (CSF) examination. The evidence concerning RA risks in MS patients is limited. Data on peripheral nerve blocks are scarce, and most data on neuraxial techniques are from the obstetric population.

Traditionally, the use of peripheral blocks in patients with MS has been regarded as safe, since blocks are performed far from the main pathogenic demyelination and scarring processes occurring within the central nervous system (e.g., 63). However, there is evidence for involvement of the peripheral nervous system in MS, with peripheral nerves showing demyelination.64 Anecdotal 65 (albeit equivocal 66, 67) reports of brachial plexopathy after peripheral nerve block have heightened awareness of the fact that a substantial share of MS patients may suffer from subclinical peripheral neuropathy, the incidence of which is reported inconsistently,68 and the clinical relevance of which remains unclear.69 Based upon circumstantial evidence, the notion that peripheral nerve blocks are most probably safe in MS patients still holds true, and peripheral RA continues to be a valid alternative to GA or central blocks.

The possibility that RA may worsen neurological status is a particular concern in spinal anesthesia. Despite the theoretical consideration that LA may have increased neurotoxic effects on demyelinated and thus pre-damaged neurons, there are no clinical studies to support this notion.70 Anecdotal evidence reports de novo exacerbation of previously undiagnosed MS through spinal anesthesia,71 but this could also be explained by transient and reversible worsening of negative symptoms through exposure to LA (see below).72 Initial reports demonstrating a higher risk of MS exacerbation after spinal anesthesia 73 have not been replicated in recent literature. A recent retrospective study found no evidence of relapse after epidural or spinal anesthesia in series of 18 and 17 patients, respectively.74
Research pertaining to neuraxial techniques in MS has been performed mostly in obstetric patients. Even though this patient population is not directly comparable to day-surgery patients, insights may be derived from these investigations. First, large-scale controlled studies have not linked any form of obstetric RA to worsening of symptoms of multiple sclerosis.\textsuperscript{70} Epidural anesthesia and analgesia, according to large-scale studies, appear safe.\textsuperscript{75, 76} Confavreux et al. reported that epidural labor analgesia was not a risk factor for disease relapse in parturients with multiple sclerosis in a trial involving 241 patients, of whom 42 had received epidural analgesia,\textsuperscript{75} confirming earlier results by Nelson and colleagues.\textsuperscript{77} This is reflected by the results of a recent survey among obstetric anesthesiologists in which the risk of epidural anesthesia / analgesia was judged to be low based on clinical experience.\textsuperscript{78} Furthermore, transient worsening of symptoms or \textit{de novo} relapse after childbirth may be mistakenly attributed to RA. After delivery, intrinsic nerve injury,\textsuperscript{79} fatigue,\textsuperscript{80} or pyrexia may exacerbate MS symptoms.\textsuperscript{78} Moreover, in patients with a relapsing form of MS, although pregnancy is associated with a decreased risk of flare-ups, the postpartum period is characterized by an increased risk of relapse. Pregnancy induces a transition from cellular immunity towards humoral immune responses, driven by the secretion of IL-10 from the feto-placental unit, thereby promoting immunological tolerance of the fetus. This cellular immunosuppression rebounds after delivery, causing increased relapse rate.\textsuperscript{75}

The limited evidence that exists is not sufficient to provide a definitive answer to the question of whether neuraxial blocks are safe in MS patients. Should symptoms arise in the perioperative period, there is an abundance of patient- and procedure-related risk factors potentially contributing to worsening of neurologic function. Moreover, the case of obstetrics illustrates that the neurological condition of the patient is not the only factor determining whether or not to perform RA. In obstetric patient, the risks of neuraxial anesthesia must
be weighed against the risks of GA in a parturient, which may explain why 90% of responders in a recent survey among obstetric anesthesiologists stated that after weighing risks and benefits, they would still perform spinal anesthesia for emergency caesarean section in MS patients.\textsuperscript{78}

Recommendations have been made to limit neuraxial doses to the lowest possible level,\textsuperscript{81} although this may increase the risk of insufficient analgesia. One argument in favor of limiting doses comes from a case-control study of 20 MS parturients, in which bupivacaine administered in doses above 0.25% was associated with what seemed to be a higher incidence of relapse.\textsuperscript{82} In general, definitive studies on pharmacological properties of LA in MS are lacking.\textsuperscript{72} Whether LA act longer on nerves from MS patients has been debated, with case reports describing normal \textsuperscript{83} versus prolonged \textsuperscript{84} block duration, and there is as yet no answer as to whether dynamics of nerve block are altered in MS. Although not tested in clinical or experimental MS settings, non-neurotoxic adjuvants such as clonidine and buprenorphine may hold promise for the future.\textsuperscript{85}

Finally, LA may directly interact with MS lesions. In patients, lidocaine can reversibly worsen symptoms of MS,\textsuperscript{86} presumably by blocking sodium channels in demyelinated areas just enough to produce symptoms, while healthy areas are not affected. These changes were noted at doses also observed systemically after epidural anesthesia in other investigations (low micromolar range).\textsuperscript{87} Paradoxically, lidocaine can worsen negative symptoms (i.e. paresis and hypesthesia resulting from demyelination), but can be used to effectively treat positive symptoms (i.e. spasticity, dysesthesia resulting from ectopic impulses) of MS, although the therapeutic range is very narrow.\textsuperscript{88} Interestingly, endogenous extracellular pentapeptides are observed in the CSF of patients with Guillain-Barre Syndrome and MS, and these pentapeptides share many electrophysiological properties with LA.\textsuperscript{89}
In summary, peripheral nerve blocks have not been proved to be harmful to MS patients, and MS should not be considered to be an absolute contraindication for neuraxial blockade. Since LA toxicity may be more pronounced in demyelinated fibers, epidural anesthesia appears safer than spinal. Overall, it seems justifiable to perform peripheral nerve blocks and epidural anesthesia in patients with MS, with due attention to restricting LA concentration. Use of spinal anesthesia in MS patients in the presence of safe and feasible alternatives should be avoided. MS patients need to be informed about potential symptom aggravation irrespective of whether RA is employed.

Post-polio syndrome

Post-polio syndrome (PPS) is the most prevalent motor neuron disease in North America. A recent review contradicted the notion that PPS affects only the aged. Indeed, many patients with PPS are of working age, and through new infections in the developing world, PPS will remain an anesthetic challenge for decades to come. PPS is characterized by central fatigue, pain and muscle weakness, frequently associated with sleep-disordered breathing (hypoventilation). Although the exact pathogenic mechanism remains obscure, current avenues of investigation implicate overuse of the enlarged motor units formed during recovery from polio, accelerated aging of motor units, persistent viral infection, and chronic inflammation.

Patients with PPS are not well suited to undergo day-case surgery because of postoperative monitoring necessities, but the sheer number of patients suffering from this disease will necessitate discussion on the perioperative use of RA, especially since these patients frequently have to undergo orthopaedic procedures. Risks of RA must be balanced against the dangers of GA, such as controversies regarding use of depolarizing and nondepolarizing muscle relaxants, sensitivity to sedative or analgesic
medication, and risk of hypoventilation and aspiration. Concerns with regard to regional anesthetic techniques include temporary deactivation of muscles that contribute to respiration during neuraxial anesthesia, difficulty in puncture during neuraxial blockade in patients with secondary abnormal spinal anatomy, and the potential of iatrogenic worsening of symptoms. Two case reports have described spinal anesthesia using normal doses of tetracaine and bupivacaine without post-procedural worsening of symptoms. Similarly, no complications were reported in a small PPS group of three patients undergoing seven surgeries. In the largest series of patients with PPS, 79 patients undergoing neuraxial anesthesia or analgesia showed no worsening of neurological symptoms.

The data provided in current literature allows no clear recommendation to choose or avoid neuraxial techniques or peripheral nerve blocks in PPS. However, there is no evidence that the performance of spinal or epidural anesthesia increases the risk of disease progression, and it seems unlikely that peripheral nerve blocks are associated with an increased risk of morbidity. Considering the unusual sensitivity towards sedative and analgesic agents, it is recommended to avoid the use of opioid or sedative adjuvants to both central and peripheral blocks.

**Guillain Barre Syndrome**

Guillain-Barre syndrome (GBS) is a heterogeneous cluster of conditions characterized by acute neuromuscular paralysis, probably due to post-infectious inflammation. In many patients, antibodies against neuronal membrane gangliosides are detectable. The disease develops weeks after the initial trigger. Paralysis is variable in extent and involvement of sensory, cranial, or autonomic nerves. The initial symptoms peak between 2 and 4 weeks after onset is followed by a much slower recovery. Diagnosis is not always straightforward,
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and is based upon clinical signs, electrophysiological and CSF diagnostics. The incidence is about 2 cases per 100,000 annually, increasing linearly with age, and predominantly affecting men.\textsuperscript{101}  GBS is not typically associated with autoimmune or systemic diseases.\textsuperscript{99}  Any individual’s prognosis is difficult to predict, and many patients continue to have moderate to severe impairment of neurologic function years after the initial onset of disease. Risk factors for prolonged neurologic deficit include older age at onset (>50 years), severe initial disease, and antecedent infection with campylobacter jejuni or cytomegalovirus.\textsuperscript{100}  The disease is usually differentiated into acute inflammatory demyelinating polyneuropathy, which features demyelinating conduction block, and acute motor axonal neuropathy, in which modifications at neuronal sodium channels are thought to play a pathogenic role. In the latter form, antibodies that act as functional blockers of sodium channels and channel phosphorylation have been implicated.\textsuperscript{100, 102}  As in MS, endogenous extracellular pentapeptides are observed in CSF (see above).\textsuperscript{89}

Reports of RA in GBS are few. The performance of peripheral nerve blocks has not been described in enough detail to allow definitive recommendations. However, in the setting of acute neuronal inflammation, peripheral blockade at these very nerves should probably be avoided. Most experience with neuraxial techniques has been gathered in obstetric patients. General comments are that the use of neuraxial anesthesia may be associated with exaggerated hypotension and bradycardia,\textsuperscript{103}  but many patients with GBS respond normally to neuraxial anesthesia.\textsuperscript{104}  Pharmacologically, both normal\textsuperscript{105}  and higher-than-normal\textsuperscript{106}  spread of LA administered using an epidural technique have been reported, but most case reports describe usual block properties.

Anecdotal evidence suggests that epidural anesthesia may activate the onset or recrudescence of GBS\textsuperscript{107, 108}  hours\textsuperscript{109}  or weeks\textsuperscript{110}  after surgery under
epidural anesthesia. The hypothetical link between RA and GBS is interaction of LA with peripheral myelin, or nerve trauma due to epidural block, with subsequent inflammation as the precipitating factor.\textsuperscript{110} However, definitive attribution of GBS to RA beyond a temporal association is difficult. Occurrence or recurrence of GBS has been described for surgery without RA,\textsuperscript{111-113} and often the time-lag between initiating trigger and onset of GBS symptoms is unclear.\textsuperscript{108}

Case reports have described successful use of epidural anesthesia at high doses, and even spinal anesthesia, in parturients with residual symptoms of GBS.\textsuperscript{105 114 115} Recently the use of combined spinal and epidural anesthesia in a parturient with GBS was reported without unusual hemodynamic effects or exaggerated effects of LA.\textsuperscript{104}

Weighing of risks and benefits of RA in patients with GBS needs to take into account the alternative risks of GA in this patient population, and the status of the disease. It has been suggested that acute neuronal inflammation should be a relative contraindication for RA\textsuperscript{116} and the possibility of a postoperative diagnostic dilemma from the combined effects of RA and GBS argue for caution. In patients with residual symptoms of GBS, the safe use of neuraxial blockade has been described using both high and low volumes and doses of LA. The few case reports describing a temporal relationship between RA and the development of GBS do not suffice to classify RA as a risk factor for GBS.

**Strategies to minimize risk in peripheral nerve blocks**

*Preoperative Assessment*

Preoperative assessment should always include a review of medical conditions that would predispose a patient to peripheral neuropathy. Subclinical peripheral neuropathy and increased vulnerability to LA toxicity may already be present in these patients. Next, documentation of existing neurologic deficits, both by history and physical examination, should be performed. Any decision to
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proceed with RA must include a risk-benefit analysis that considers the possible increased risk of peripheral nerve blockade in patients with a peripheral neuropathy compared with the risks of GA in the same patient. Importantly, the risks and benefits of RA should be discussed with the patient and documented in the medical record. Recent surveys of regional anesthesiologists both in academic and community settings have suggested that this practice is not consistently performed.117

Technique of Regional Anesthetic

Choice of nerve localization technique and its relation, if any, to the risk of peripheral neuropathy has long been a topic of controversy. The goal of nerve and needle localization has traditionally been to deposit the LA around the epineurium without penetration or laceration, using nerve stimulation. One limitation of stimulator techniques is the lack of a predictable relationship between the threshold for nerve stimulation and needle-to-nerve distance. Significant variability exists between patients and for different nerve localization sites. This relationship may even further be complicated in patients with underlying neuropathies, such as DPN.55 Ultrasound guidance may improve needle placement accuracy and reduce LA dose,58 which are both desirable in neuropathic patients. While this seems plausible and of particular interest in patients with neuropathy, superior safety by using ultrasound imaging has yet to be confirmed.118

Choice of Local Anesthetic

A second area of controversy is type and concentration of LAs. All LAs are neurotoxic at high concentrations,119 and potentially toxic even at clinical concentrations.85 Neurotoxic effects are comparable between LAs at equipotent doses. Diabetic nerves in animal studies have demonstrated an increased
sensitivity to LAs. Diabetic nerves require a lower concentration of LA for successful nerve block and have a longer duration of block. Concentration of LA and duration of nerve block correlate with histological fiber damage. It is noteworthy that in Kalichman’s study of sciatic nerves of diabetic rats, significantly increased nerve fiber damage was noted at 4% lidocaine versus 2% lidocaine. In the study by Kroin et al, 1% lidocaine caused no significant nerve fiber injury in diabetic rats. Thus, use of the lowest effective concentration and amount of LA may reduce the risk of nerve fiber damage. This raises the question of what concentration of LA is necessary for adequate surgical anesthesia for, e.g., DPN patients. A recent study by Kocum and colleagues showed that 0.25 % bupivacaine may be employed to produce effective femoral and sciatic blocks in patients with diabetic foot syndrome. Further investigations are needed to determine the optimal concentration of LA that achieves successful surgical anesthesia while minimizing risk.

Role of Adjuvants

Epinephrine reduces blood flow in the peripheral nerve. Although the temporary reduction in blood flow is well tolerated by most peripheral nerves, the nerves of patients with compromised vascular integrity due to diabetes, arteriosclerosis, or other injury may not tolerate severe reductions in blood flow. Furthermore, by reducing neural blood flow, epinephrine decreases the washout of LA and prolongs the duration of LA exposure. These properties prolong the LA block for intermediate acting LA and may potentiate toxicity. In diabetic nerves, epinephrine 5 mcg/ml prolongs the block by lidocaine 1%, causing histologic evidence of axonal degeneration, which is absent with lidocaine or epinephrine alone. In another study, epinephrine 5 mcg/ml caused significant reduction in blood flow of healthy nerves, whereas epinephrine 2.5 mcg/ml did not. On this basis, some have advocated using reduced doses of epinephrine in
patients with peripheral neuropathy to reduce the risk of nerve damage. \textsuperscript{118,123} We advocate a more conservative stance of avoiding epinephrine in DPN patients altogether. The absence of this marker for intravascular injection, we also recommend slow, incremental administration of the LA dose, and use of lidocaine in preference to agents capable of producing malignant ventricular dysrhythmias such as bupivacaine and ropivacaine. \textsuperscript{124,125} Similarly, clonidine has been advocated as a useful adjuvant for peripheral nerve blockade. \textsuperscript{123} In doses as low as 0.1 mcg/kg, clonidine has been shown to significantly increase the duration of anesthesia for intermediate acting LA. \textsuperscript{126} The effect of clonidine is peripherally mediated and is likely the result of activity dependent hyperpolarization of the peripheral nerve fibers. \textsuperscript{127} In diabetic sciatic nerves, the addition of clonidine 7.5 mcg/ml to 1% lidocaine significantly prolonged block duration compared with lidocaine 1% plain but also produced histologic evidence of axonal degeneration. \textsuperscript{44} Of note, this represents a higher dose of clonidine than would be used clinically. Further investigations using lower concentrations of clonidine may reveal a dose where anesthesia may be augmented without augmenting axonal toxicity. \textsuperscript{128} Clonidine and buprenorphine \textsuperscript{85} may prove to be useful adjuvants or replacements \textsuperscript{129} for LA.

\textit{Summary}

Based on limited data, mostly from animal studies, the risk of axonal injury to peripheral nerves compromised by peripheral neuropathy may be reduced by limiting the concentration and duration of LA exposure. Strategies to accomplish this may include reducing the concentration of the selected LA, reducing or avoiding added epinephrine, and utilizing a nerve localization technique that minimizes the incidence of intraneural injections. Further investigations should clarify the safe and effective concentrations of LA and adjuvants in patients with peripheral neuropathy, and whether the potential for
combined adjuvants reduces or eliminates the need for LA. Similarly, the safety of different nerve localization techniques and nerve block selection should be explored to optimize safety in this vulnerable, and growing patient population.

**Strategies to minimize risk in neuraxial blocks**

*Evaluation of Risk*

Several authors have speculated that patients with peripheral neuropathy may be more vulnerable to symptomatic complications after neuraxial block. First, due to the “double-crush” phenomenon, otherwise asymptomatic lesions along a conduction pathway may produce symptoms following even a mild degree of anesthetic-induced neurotoxicity. Second, “peripheral neuropathies” such as diabetic polyneuropathy, may also produce changes in the spinal cord.

*Choice of Local Anesthetic*

Similarly to effects on the peripheral nervous system, all LA can cause dose dependent neurotoxicity. Some studies have raised concern that lidocaine in clinically used concentrations may have a propensity for increased neurotoxicity compared with similarly potent doses of bupivacaine. The presence of peripheral neuropathy has not been associated with transient neurologic symptoms in ambulatory patients. Limiting the dose and concentration of LA in neuraxial blocks has been advocated to minimize the risk of neurotoxicity. In ambulatory patients, the use of the lowest effective dose of LA may also enable rapid return of sensory and motor function.

*Use of Adjuvants*

Hydrophobic opioids such as fentanyl have been added to both low dose bupivacaine and lidocaine spinal anesthetics to augment sensory blockade. A relative paucity of animal data exists regarding the toxicity of spinal fentanyl.
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In clinical practice, few concerns about neurotoxicity have been raised regarding low dose fentanyl or sufentanil used for ambulatory anesthesia. The limited animal studies which have been done have not revealed significant histologic or physiologic evidence of neurotoxicity. Therefore the addition of lipophilic opioids to low doses of LA appears to be a useful adjunct of to decrease the total dose of LA needed for successful surgical anesthesia in ambulatory patients, but with the concomitant undesirable side-effects such as pruritus, nausea, and somnolence in the elderly.

Epinephrine is rarely used in ambulatory patients as the resulting delayed resolution of neuraxial anesthesia is usually not desirable for ambulatory patients undergoing relatively short procedures. Epinephrine in most clinically used doses does not significantly affect spinal cord blood flow. However, epinephrine does worsen spinal cord injury when given with 5 % lidocaine or 1 to 2 % tetracaine in rats. It is likely that this effect is due to reduced clearance and therefore prolongs exposure to the LA than to any direct effect of the epinephrine. Also, when used with chloroprocaine, epinephrine can cause flu-like symptoms. Because of its undesirable prolongation of anesthesia and possible enhancement of toxicity, neuraxial epinephrine is best avoided in patients with peripheral neuropathy presenting for ambulatory anesthesia.

Summary

Neuraxial anesthesia can be provided to patients with preexisting neuropathy on the basis of thorough risk-benefit-analysis. Strategies to minimize the risk of nerve toxicity include the use of the lowest effective dose of LA, avoidance of epinephrine, and possible use of adjuncts such as lipophilic opioids. Careful selection of agents can provide optimal surgical anesthesia and time to discharge readiness as well as maximizing safety.
Conclusion

RA is a reasonably safe and at the same time highly effective method of providing pain relief to day-surgery patients. There is no universal answer to the question of whether a regional anesthetic technique is the best choice for patients with preexisting neuropathy. In most patients, the decision is complex and requires a careful and individual weighing of risks and benefits of both general anesthesia and regional anesthesia. In patients with preexisting neurological disease, function can deteriorate perioperatively irrespective of whether RA is employed. In diseases such as DPN and MS, reducing local anesthetic concentration is appropriate. Focused informed consent regarding RA versus GA with these patients should be followed by close observation for potential sequelae.

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### Table 1
Proposal for concentration and maximum single dose of local anesthetics in patients with DPN based upon equipotency.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>DPN-adjusted</th>
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<tbody>
<tr>
<td>Lidocaine</td>
<td>2 %</td>
<td>1 %</td>
</tr>
<tr>
<td>Mepivacaine</td>
<td>1.5 %</td>
<td>0.75 %</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>0.75 %</td>
<td>0.375 %</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>0.5 %</td>
<td>0.25 %</td>
</tr>
</tbody>
</table>

Equipotency values based on Hadzic A (ed.) Textbook of Regional Anesthesia and Acute Pain Management, 2006, The McGraw-Hill Companies. We are indebted to Brian Williams MD, MBA, for his kind permission to use his calculations in this manuscript.
Effects of subclinical diabetic neuropathy on sciatic nerve block

Based on
Subclinical Diabetic Neuropathy Increases In Vivo Lidocaine Block Duration But Not In Vitro Neurotoxicity.
Introduction

Local anesthetics are widely used to block nerve conduction for surgical anesthesia, or to manage acute and chronic pain. Unfortunately, local tissue damage, in particular neurotoxicity induced by local anesthetics, remains a substantial concern. This concern may apply even more to nerves with pre-existing pathology. Neuropathy due to diabetes mellitus (DM) may be a relevant risk factor for regional anesthetic procedures. It is the most common neuropathy worldwide, affecting a large proportion of diabetes patients. The relevance of diabetic neuropathy for daily anesthetic practice lies in the fact that because of their frequent comorbidities, diabetic patients are predestined to undergo many types of surgery under regional anesthesia. Results from some experimental trials suggest that diabetic neuropathic nerves are more susceptible to local anesthetic-induced neurotoxicity than healthy control nerves during peripheral nerve blockade, while another recent trial found no difference in toxicity when effects of spinal anesthesia were examined. Epidemiological data suggest that nerve damage following regional anesthesia may be up to ten times more frequent in diabetic patients as compared to the general patient population. The effect of isolated hyperglycemia on local anesthetic-induced neurotoxicity has not been investigated.

According to the “double-crush-hypothesis”, pre-existing nerve damage may further predispose nerves to suffer from local anesthetic-induced neurotoxicity. One pathogenic pathway activated in both diabetic neuropathy and local anesthetic-induced neuropathy is activation of the p38 Mitogen-Activated Protein Kinase (MAPK). Neurotoxicity of the prototype local anesthetic, lidocaine, is mediated by specific activation of the p38 MAPK, while its inhibition significantly attenuates toxicity. Similarly, expression of the p38 MAPK has been correlated with the development of diabetic sensorimotor neuropathy in a streptozotocin-induced rat diabetic model, while its inhibition resulted in restoration of normal nerve conduction.

Further, it has been controversially discussed whether diabetic peripheral neuropathy affects block duration. Three studies investigated this in a streptozotocin-induced rat model, with one study demonstrating normal, and two
studies showing prolonged block duration.\textsuperscript{7, 8} In diabetic patients, spinal anesthesia has been reported to last longer than in healthy control patients.\textsuperscript{13}

The present study therefore aimed to test in a rat model of Type II diabetes, the hypothesis that pre-existing subclinical neuropathy, but not isolated hyperglycemia, would predispose nerves to injury by local anesthetics. Our secondary goals were to determine length of nerve block, and examine potential neuroprotective effects of an inhibitor of the p38 MAPK, SB 203580.

**Methods**

Experimental procedures were approved by the Animal Care and Use Committee of the Austrian Federal Ministry of Science, Education and Culture (BMWF-66.011/0097-C/GT/2007) and the Harvard Medical Area Standing Committee on Animals (Boston, MA).

**Drugs**

For *in vitro* experiments, the pH of the lidocaine stock solution 1 M was 4.65 (in dimethylsulfoxide). The pH of the final solution added to medium was 7.38 for lidocaine 40 mM (Sigma Aldrich, Vienna, Austria), which corresponds to a concentration of approximately 1%. The concentration of dimethylsulfoxide (Sigma, Vienna) in treated cultures was not significantly higher than in control cultures incubated with vehicle only. The inhibitor of the p38 MAPK, SB203580, was co-incubated with lidocaine in selected cultures at a final concentration of 10 \( \mu \text{M} \). D-Glucose (Sigma, Vienna) was prepared in a 1 M stock solution in distilled water. For experiments, we diluted glucose in Roswell Park Memorial Institute (RPMI)-medium supplemented with B27- and antibiotic additives to achieve a final concentration in medium of 5 mM (control) or 25 mM (hyperglycaemic).\textsuperscript{14} Incubation of neuronal cell cultures with lidocaine, with or without addition of SB203580 (Sigma, Vienna), was performed for 24 hours. This timeframe was used in previous investigations and, combined with the lidocaine concentration of 40 mM, resulted in approximately 50% of cell death.\textsuperscript{11, 15, 16}
Animals

We employed adult male Zucker Diabetic Fatty (ZDF) rats, a combined congenital / dietary inbred model of type II diabetes mellitus obtained from Charles River Laboratories (Sulzfeld, Germany or Wilmington, MA). The main pathogenic mechanism in these animals is a homozygous missense mutation in the leptin receptor (ZDF<sup>fa/fa</sup> genotype). ZDF rats develop metabolic syndrome, including hyperglycemia. Two types of ZDF rats were used: the genotype homozygous for obesity and metabolic syndrome (ZDF<sup>fa/ha</sup>, “diabetic”), and heterozygous normoglycemic animals (ZDF<sup>fa/-</sup>, “control”). The progression from pre-diabetic to diabetic state has been described in detail. Diabetic rats suffer from obesity, insulin resistance, and overt type II diabetes mellitus starting at 7 to 10 weeks of age. At age 12-14 weeks, ZDF rats develop slowing of conduction velocity indicative of diabetic neuropathy. Animals were used at 6 weeks of age for cell culture experiments into acute and chronic hyperglycemia, and at 12 weeks of age for in vitro and in vivo experiments into subclinical diabetic neuropathy. Until experiments, diabetic rats received water and irradiated Purina diet #5008 (Charles River Laboratories) <em>ad libitum</em>. This diet has been shown to most reliably promote the progression of metabolic syndrome in ZDF rats and has been used in previous investigations on diabetic neuropathy in this model, e.g.,

Primary sensory neuron culture

Dorsal root ganglion (DRG) cultures were obtained as described previously. Neurons were acutely harvested from (ZDF<sup>fa/ha</sup>) or ZDF control rats, which were euthanized by carbon dioxide narcosis according to institutional protocol (Animal Care and Use Committee of the Austrian Federal Ministry of Education, Science and Culture, Vienna, Austria). DRGs were de-sheathed and incubated in collagenase 5000 U/ml for 90 min at 37°C, followed by 15 min in 0.25% trypsin/EDTA. After dissociation in Roswell Park Memorial Institute (RPMI) medium containing 10% horse/5% fetal bovine serum, neurons were plated in RPMI medium supplemented with B27 additives (1:100) and antibiotics (penicillin, 1000 units/ml; streptomycin, 1000 µg/ml; and amphotericin B, 25 µg/ml in 0.85% saline), all purchased from Invitrogen (Carlsbad, CA). Neurons were allowed to adhere to
the glass floor of dishes coated with poly-D-lysine/laminin for 24 h. Poly-D-lysine was applied at a concentration of 0.1 mg/ml in distilled H$_2$O and laminin at 7 µg/ml in RPMI solution. Cell cultures were kept at 37°C in a humidified atmosphere containing 5% CO$_2$.

**In vitro model of diabetic metabolism**

After harvesting ganglions from healthy ZDF$^{fa/-}$ control rats, cell cultures had 24 hours time to stabilize until continued treatment with glucose. Subsequently, we maintained control (5 mM) and high (25 mM) glucose concentrations for 24 or 72 hours to simulate acute or hyperglycemia, or diabetic metabolism as described before.  

**Inhibition of p38 MAP kinase**

To determine whether blocking of p38 MAPK would increase neuronal cell survival in neurons treated with lidocaine, we co-incubated some cultures with an inhibitor of the p38 MAPK, SB203580, at a concentration of 10µm.

**Quantification of neurotoxicity in vitro**

We quantified neurotoxicity using the cytotoxicity detection kit (Roche Diagnostics, Graz, Austria). This assay detects lactate dehydrogenase (LDH) released from injured cells. We used sterile 96-well tissue culture plates, and incubated cells as described above with experimental drugs. Cell injury results in release of LDH, which is subsequently present in the culture supernatant. Supernatant is extracted and mixed with reagent containing a tetrazolium salt, which is cleaved by LDH to result in the formation of formazan after incubation at 30 minutes at room temperature under light protection. Detection of water-soluble formazan is done by determining absorption at 492 nm, interpolating values with low and high controls included in the kit. Plausibility control was undertaken to remove extreme outliers. Due to measurement variability, some LDH values resulted in survival values of just above 100%.
Peripheral nerve blockade

In vivo experiments were conducted according to a protocol approved by the Harvard Medical Area Standing Committee on Animals (Boston, MA). Neurobehavioral investigation was performed as described before by an investigator blinded to experimental group allocation. We performed in vivo experiments on ZDF\textsuperscript{fa/fa} ("diabetic") and ZDF\textsuperscript{fa/-} ("control") rats. All rats were weighed before experiments. We tested nociceptive and sensory function with mechanical pinch with serrated forceps. Tests were performed bilaterally at all measurement time points. In male diabetic ZDF animals, a specialized diet such as applied in our animals results in hyperlipidemia and hyperglycemia by 8 weeks of age, and diabetes by 12 weeks of age (Charles River Laboratories). In functional tests, motor nerve conduction velocity as a sign of subclinical diabetic neuropathy is decreased at 12-14 weeks of age, indicating first underlying pathological processes.\textsuperscript{19}

Test animals were anesthetized with Sevoflurane (Abbott, Abbott Park, IL) during all procedures. Sciatic nerves were surgically exposed by lateral incision of the thighs and division of the superficial fascia and muscle as described before.\textsuperscript{21} A volume of 0.2-mL test drug was injected directly beneath the clear fascia surrounding the nerve but outside the perineurium, proximal to the sciatic bifurcation. We chose lidocaine 2% (corresponding to approximately 80 mM) as described previously.\textsuperscript{21} The wound was sutured with 3-0 vicryl suture.

Motor function was assayed before injection, and after 15, 30, 45, 60, 75, 90, 120, and 150 minutes by holding the rat upright with the control hind limb extended so that the distal metatarsus and toes of the target leg supported the animal’s weight; the extensor postural thrust was recorded as the force (in grams) applied by each of the two hind limbs to a digital platform balance (Ohaus Lopro; Fisher Scientific, Florham Park, NJ). The reduction in this force, representing reduced extensor muscle contraction caused by motor block, was calculated as a percentage of the control force (preinjection control value 90–130 g). The percent reduction in force was assigned a “range” score: 0 = no block (or baseline); 1 = minimal block, force between the preinjection control value of 100% and 50%; 2 = moderate block, force between 50% of the preinjection control value and 12 g
(approximately 12 g represented the approximate weight of the flaccid limb); 3 = complete block, force 12 g or less.

Nociception was evaluated by the nocifensive withdrawal reflex and vocalization to pinch of a skin fold over the lateral metatarsus (cutaneous pain) with a serrated forceps; the force and duration of this pinch was held as constant as possible. The extent of the nocifensive withdrawal reflex and vocalization were combined on a scale of 0–3 for each examination. Grading was as follows: 3 = complete block, no nocifensive reaction or vocalization; 2 = moderate block, vocalization accompanied by slow withdrawal or flexion of the leg; 1 = minimal block, brisk flexion of the leg, with some sideways movement of the body or other escape response and loud vocalization; 0 = baseline with quick nocifensive responses listed above. The contralateral limb was used as control in all experiments. We defined the time to complete absence of block (motor block and nociceptive block score = 0) as block time.

Statistics

We used ANOVA and if significant unpaired t-test to compare data between two groups. Statistical significance was assumed at $P < 0.05$. Bonferroni-Holm was used to correct for multiple group comparisons. SPSS 16.0 was used for statistical analyses.

Results

In vitro, acute or chronic hyperglycemia do not increase lidocaine neurotoxicity

The application of control or high concentrations of glucose for 24 hours prior to lidocaine incubation did not alter neurotoxicity. In comparison to control cultures and high glucose alone, incubation with lidocaine alone led to a reduction in survival of about 50% in both control and high-glucose cultures (Table 1). Co-application of SB203580 with lidocaine did not prevent lidocaine-induced neurotoxicity (52 ± 15%, n = 16).

In both cultures incubated with control medium or high glucose levels for 72 hours, lidocaine incubation led to a reduction in cell survival of approximately
50% (Table 1). Co-application of SB203580 with lidocaine resulted in a cell number of 50 ± 25% (n = 16, non-significant).

**In vitro, lidocaine is more neurotoxic in diabetic than in control cells, and co-incubation with SB203580 reduced neurotoxicity**

In neuron cultures from diabetic animals harvested at 12 weeks of age, control cultures had a viability of 117 ± 7 % (n = 18). Cultures from control animals incubated with lidocaine had a cell count of 64 ± 9 % (n = 33, \( P < 0.001 \) as compared to controls), whereas neurons from diabetic animals had a cell count of 57 ± 19 % (n = 64, \( P < 0.001 \) as compared to controls, \( P < 0.05 \) as compared to non-diabetic animals after multiple-group correction, Figure 1).

Neurons from diabetic animals incubated with lidocaine had a survival rate of 57 ± 19 % (n = 64), and addition of SB203580 to lidocaine in neurons from diabetic animals resulted in a survival of 71 ± 12 % (n = 66, \( P < 0.001 \) to controls, \( P < 0.001 \) as compared to lidocaine in diabetic animals). Some non-diabetic cultures were incubated with lidocaine and SB203580, resulting in a survival of 66 ± 9 % (n = 33, non-significant as compared to lidocaine in diabetic animals).

**In vivo, subclinical diabetic neuropathy prolongs lidocaine duration of action**

At baseline, behavioural tests were not significantly different between diabetic and non-diabetic rats. In detail, motor testing revealed a force of 111 ± 12 g in control and 114 ± 12 g in diabetic animals. Nociceptive testing was 0 in all control, and 0 in all diabetic animals. In diabetic animals, motor block lasted significantly longer than in control animals (137 ± 16 min versus 86 ± 17 min, \( P < 0.001 \), Figure 2A). Response to deep pinch was abolished longer in diabetic than in non-diabetic rats (128 ± 22 min versus 77 ± 10 min, \( P < 0.001 \), Figure 2B). Response to superficial pinch was similarly abolished longer in diabetic than in non-diabetic rats (128 ± 22 min versus 81 ± 8 min, \( P < 0.001 \), Figure 2C). All animals regained the values of the baseline behavioural test within a day. Return to baseline values for neurobehavioral tests was observed in all animals.
Discussion

The main findings of our study are that (A) in vitro, lidocaine neurotoxicity is not altered by acute (24h) or chronic (72h) hyperglycemia; (B) in vitro, despite reaching statistical significance, subclinical diabetic neuropathy does not appear to substantially increase lidocaine neurotoxicity; (C) in vitro, inhibition of p38 MAPK, at least partly, reversed lidocaine neurotoxicity in diabetic neurons; and (D) in vivo, subclinical diabetic neuropathy increases block duration without apparent adverse behavioral effects after the blocks resolve. However, we did not histologically examine these lidocaine-exposed sciatic nerves to address potential disruption of fiber integrity after behavioral tests had returned to baseline. In nerve cell cultures from non-diabetic animals, lidocaine resulted in a reduction in cell survival of about 50%, confirming results from previous investigations and corroborating the experimental in vitro paradigm.11 21

In vitro, acute or chronic hyperglycemia do not increase lidocaine neurotoxicity

We first sought to determine whether acute or prolonged hyperglycemia per se would be sufficient to increase lidocaine neurotoxicity in vitro. Hyperglycemia alone did not enhance lidocaine-induced neurotoxicity. Even though a period of 72 hours has previously been described as sufficient to elicit first general changes indicative of diabetic metabolism in PC12 cell line cultures,14 this approach may not result in long-standing alterations characteristic of severe diabetic neuropathy which may predispose nerves to injury following exposure to local anesthetics. Using doses of glucose which reflect previous investigations seeking to simulate diabetic conditions in vitro,14 we were unable to detect a negative impact of acute or chronic hyperglycemia on local anesthetic neurotoxicity.

In vitro, lidocaine neurotoxicity is comparable between diabetic and control cells

Secondly, neurons explanted from animals with longer-lasting diabetes (12 weeks) were compared to neurons harvested from non-diabetic animals with regards to sensitivity towards lidocaine neurotoxicity. The slight survival advantage of non-diabetic neurons observed was small, and, despite reaching statistical significance, appears not to reflect clinically relevant neurotoxicity. No previous investigations
have investigated the potential of neurotoxicity in neurons explanted from a genetically modified rodent model. The timing of neuron harvesting was chosen to precede development of manifest diabetic neuropathy. It is generally thought that diabetic nerves are more susceptible to exogeneous trauma / ischemia / toxins. However, the results obtained by us are not as pronounced as those reported previously in vivo by others. Two reasons may account for this discrepancy. Firstly, at the time of harvesting, animals had long-standing hyperglycemia, but presumably only mild subclinical neuropathy, while animals in other trials were pronounced neuropathic. Zucker rats reliably develop progressive neuropathy, so by choosing a later timepoint for experiments, we may have found a more pronounced neurotoxic effect. Also, a recent study in Type I diabetic rats subjected to repeated intrathecal injections failed to demonstrate significant neurotoxicity. Secondly, the model employed by us is a model of DM type II, reflecting metabolic syndrome rather than the streptozotocin (STZ) model used in previous investigations. It has been stated that there is no single best animal model for research into regional anesthesia in diabetic neuropathy. We believe the model chosen by us relates reasonably well to the clinical situation, in which the majority of neuropathic patients presenting for surgery are type II diabetics.

In vitro, co-incubation of lidocaine with SB203580 reduces neurotoxicity in diabetic neurons

Physiologically, the p38 MAPK is a member of the family of mitogen-activated protein kinases, key regulators of eukaryotic cell regulation. p38 MAPK is activated following a broad variety of stressors such as environmental factors (cytotoxic substances, radiation, osmotic stress, heat shock), inflammatory cytokines (e.g. Tumor Necrosis Factor α), and growth factors. P38 MAPK potently activates transcription factors and other protein kinases. Therapeutic inhibition of p38 MAPK activity has been shown to be of potential benefit in, among others, experimental nerve trauma, excitotoxicity, and growth factor withdrawal.

In the setting of diabetic neuropathy, activation of p38 MAPK (leading to neuronal degeneration) coincides with the development of functional deficits in a STZ-based rat model, while pharmacologic inhibition of MAPK greatly attenuates
functional changes. Similarly, p38 MAPK activation occurs when primary sensory neurons are exposed to toxic doses of local anaesthetics, and inhibition of p38 MAPK leads to decreased neurotoxicity in vitro and in vivo. We therefore hypothesized that activation of the same neurodegenerative p38 MAPK pathway by both diabetic neuropathy and local anaesthetic aggravates neuronal damage. This should be most prominent when lidocaine is applied, because the latter selectively activates p38 MAPK in neurons. We found only minimally increased neurotoxicity when lidocaine was applied to nerves harvested from diabetic animals as compared to control animals. Inhibition of the p38 MAPK, at least in part, abolished this effect in vitro. Inhibition of p38 MAPK reduced the lidocaine-induced neurotoxicity in diabetic neurons, confirming our experimental hypothesis. Effects on healthy neurons were not statistically different. The protective effect of p38 MAPK in healthy neurons was smaller compared to previous investigations. Several factors may account for the unexpected finding that lidocaine neurotoxicity in control cells was unaffected by p38 MAPK inhibition. The model used in this study differs from that used in previous studies as it represents a different genotype of rats (ZDF versus Sprague Dawley rats or PC12 cell line cultures).

In vivo, subclinical diabetic neuropathy prolongs lidocaine duration of action

We next investigated influence of diabetic subclinical neuropathy upon lidocaine block duration. At baseline, we found no difference in sensitivity, supporting our model of subclinical diabetic neuropathy. When lidocaine was applied to diabetic nerves, it led to a longer-lasting sensory and motor block compared to wild-type controls. In a previous study using the STZ diabetes model, Kalichman and Calcutt showed that lidocaine exhibited normal duration of block in diabetic nerves. Our results are, however, in concordance with a recent investigation by Kroin et al., who demonstrated increased sciatic nerve block duration in STZ diabetic rats, using a slightly longer pretreatment time. In the same STZ experimental model in rats, spinal anesthesia was reported to result in a longer block duration in diabetic animals without a concomitant increase in neurotoxicity. In diabetic patients, spinal anesthesia was reported to last longer than in non-diabetic patients.
The reason for increased block duration may be multifactorial, involving changes in nerve metabolism and physiology, and nerve fiber damage. Previous investigations had used open or percutaneous nerve block. We cannot exclude that the open approach resulted in inflammation around the nerve fascia, but in a recent experimental study, Kroin et al. found no difference in block duration whether nerve block was performed open or closed.

**Limitations**

Some limitations of the present study should be briefly addressed. First, our in vitro data cannot be directly correlated with clinical practice. As for peripheral nerve blockade (rat sciatic nerve block model), the drugs are applied to the axon rather than the DRG, making dissociated neuronal cultures an imperfect approximation. Moreover, glial cells, usually found in standard DRG cultures, could influence LA-induced neurotoxicity. We used lidocaine as the prototype amide-type local anesthetic. For peripheral nerve block in clinical practice, other drugs such as mepivacaine (short-acting) or long-acting local anesthetics are more frequently used. We suggest the ZDF model to be used to investigate other local anesthetics in regular clinical use, and to include a group of animals with even longer-standing diabetes to evaluate fully developed diabetic neuropathy, as well.

The ZDF rat has been described as a model of type II diabetes mellitus, but features other conditions as well, most notably obesity. In the ZDF model, male rats develop manifest hyperglycemia at 7 weeks of age. At the age of 12-14 weeks (the timepoint used for our experiments), ZDF rats feature an overt neuropathic deficit discernible by electrophysiologic investigation. We attest to the fact that we did not confirm these investigations in our test animals, such that presence of subclinical nerve damage was inferred from the literature showing a predictable clinical course of diabetes and complications, and a definable window when this damage appears. The ZDF model chosen to investigate the effects of long-standing hyperglycemia on regional anesthesia has been widely used in metabolic research. Nevertheless, a recent editorial highlighted the fact that all current diabetes models feature confounding factors. In the case of ZDF, it has been argued that it is more a model of metabolic syndrome than exclusively for diabetes, while the same applies
for STZ-induced diabetes Type I, which is confounded by hepatic and renal damage, and substantial behavioural changes. Our model is most likely more representative of clinical reality for anesthesiologists, in which patients with neuropathy due to Type II diabetes represent the most controversial patient collective.

Finally, we concluded from behavioural data that no overt neurotoxicity was observed in our model, without performing neurohistopathological investigations once tests had returned to baseline. Future investigations should include histological examination to corroborate our findings. We did not carry out neurobehavioural testing after tests had returned to baseline.

**Conclusion**

In conclusion, we demonstrate that *in vitro*, acute or chronic hyperglycemia per se does not increase lidocaine-induced neurotoxicity. We observed a small, albeit statistically significant, increase in neurotoxicity when lidocaine was applied to diabetic as compared to non-diabetic neurons, but the difference in our model appears small. The inhibitor of the p38 MAPK, SB203580, can at least partially reverse lidocaine-induced neurotoxicity in diabetic nerves. *In vivo*, block duration is substantially prolonged by subclinical diabetic neuropathy.

**References**

Regional anesthesia in subclinical neuropathy


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Chapter 3.3


### Table 1
Survival in response to lidocaine after acute or chronic hyperglycemia

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Survival</th>
<th>n</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute hyperglycemia (24 hours)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>105 ± 4</td>
<td>9</td>
<td>S vs. 2 and 4</td>
</tr>
<tr>
<td>2. Lidocaine</td>
<td>45 ±15</td>
<td>17</td>
<td>S vs. 1 and 3</td>
</tr>
<tr>
<td>3. High glucose</td>
<td>107 ± 6</td>
<td>17</td>
<td>S vs. 2 and 4</td>
</tr>
<tr>
<td>4. High glucose plus lidocaine</td>
<td>55 ±13</td>
<td>17</td>
<td>S vs. 1 and 3</td>
</tr>
<tr>
<td><strong>Chronic hyperglycemia (72 hours)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>103 ± 4</td>
<td>9</td>
<td>S vs. 2 and 4</td>
</tr>
<tr>
<td>2. Lidocaine (as above)</td>
<td>45 ± 15</td>
<td>17</td>
<td>S vs. 1 and 3</td>
</tr>
<tr>
<td>3. High glucose</td>
<td>102 ±7</td>
<td>17</td>
<td>S vs. 2 and 4</td>
</tr>
<tr>
<td>4. High glucose plus lidocaine</td>
<td>53 ± 27</td>
<td>16</td>
<td>S vs. 1 and 3</td>
</tr>
</tbody>
</table>

Legend: S significant NS non-significant.
Figure legends

Figure 1. Comparison of viability of dorsal root ganglia primary cultures of diabetic (ZDF\textsuperscript{fa/ta}) and control (ZDF\textsuperscript{fa/-}) animals. Cells were exposed for 24 h to control medium, lidocaine or lidocaine and MAP kinase inhibitor SB203580. Data are displayed as mean and standard deviation. Comparisons were made by means of student-t-test with Bonferroni-Holm correction. * delineates p < 0.0001; ‡ = p < 0.01; # = p < 0.05.

Figure 2. The time course of motor (A) and deep sensory (B) block in control and diabetic animals is displayed during 150 min after injection of lidocaine. Predefined four-point scales were chosen with 0 denoting no block and 3 complete block. Data presented as mean ± standard error, testing was done by means of ANOVA with posthoc Benforoni test. * denotes p < 0.05.
Note: For all nerve conduction tested a significantly prolonged block was seen in diabetic animals.
Figure 2

A. Motor block

B. Deep pinch sensation

Legend:
- Control
- Diabetic
Effects of early and diabetic neuropathy on sciatic nerve block duration and toxicity

Based on
Effects of early and late diabetic neuropathy on sciatic nerve block duration and neurotoxicity.
Introduction

Diabetic peripheral neuropathy (DPN) is a frequent complication of both Type I and Type II diabetes mellitus (DM), and the most prevalent neuropathy in the Western world.\(^1\) Diabetics undergo surgery more often than non-diabetic patients,\(^2\) and several surgical procedures for typical complications of long-standing DM, e.g. creation of arteriovenous fistula in patients with end-stage renal disease, might be preferably performed under regional anaesthesia.\(^3\)

However, diabetic neuropathic nerves may be more sensitive to local anesthetics and their toxicity, and this hypothesis is supported by two lines of evidence. Firstly, regional anaesthesia in diabetic neuropathic patients may be associated with increased risk of neurological injury.\(^4\) Limited epidemiological evidence suggests higher risk of neurotoxicity in diabetic neuropathic patients,\(^5,6\) even if experimental evidence has been equivocal.\(^7\) Secondly, DPN may influence nerve block duration.\(^4\) Clinical\(^8,9\) and experimental\(^10,11\) evidence suggests that block duration may be prolonged in diabetic neuropathic nerves. However, most studies were carried out in models of streptozotocin-induced Type I DM, which does not reflect clinical reality, in which the huge majority of patients suffer from Type II DM.\(^5\)

Our aim was to determine the impact of regional anaesthesia in DPN in an animal model for Type II DM. We therefore sought to devise a comprehensive model using behavioural, electrophysiological, and histopathological investigations to determine neurotoxicity of a lidocaine 2\% peripheral nerve block and duration of this nerve block in Zucker Diabetic Fatty rats with early diabetic neuropathy, advanced diabetic neuropathy, and advanced diabetic neuropathy under partial glycemic control. Our working hypothesis was that in a rodent model of Type II DM, the presence of advanced (18 weeks) but not early (10 weeks) neuropathy would lead to increased neurotoxicity and block duration following sciatic nerve block with lidocaine as compared to age-matched healthy control animals. The main endpoint was neurohistopathology one week after nerve block.
Materials and Methods

The present study protocol was approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, protocol number LEICA102868-1. Methods and results are reported according to ARRIVE guidelines.12

Animals

Experiments were carried out on the left sciatic nerve of Zucker diabetic fatty (ZDF) rats, obtained from Charles River Laboratories (L’Arbresle, France). This inbred model of Type II DM combines a genetic predisposition (homozygous leptin receptor mutation fa/fa, “diabetic”, or heterozygous mutation fa/+, “control”) with a dietetic component (Purina #5008 diet, Charles River, L’Arbresle, France).11 Animals were obtained at 9 weeks of age and were given one week for acclimatization. For all electrophysiological measurements, sciatic nerve block, and placement of insulin release implants, animals were anaesthetized using isoflurane (Baxter, Utrecht, The Netherlands) with an inspiratory concentration between 2 – 3 Vol%, since this regimen least affects electrophysiological measurements in rodent models.14 Adequacy of anaesthesia was ascertained using sensory testing. We only carried out procedures when the animal did not react to a forceful pinch to the forefoot using a forceps. All procedures were performed percutaneously to minimize animal distress, and analgesic rescue was buprenorphine 0.05 mg/kg body weight. Detailed welfare assessment concerning appearance and behaviour was carried out at least weekly by an animal care technician unrelated to the experiment. After the last measurements, while still under isoflurane anaesthesia, animals were euthanized using CO₂ narcosis.

Experimental groups

The timeline of experimental procedures is given in Figure 1. In all experimental groups, baseline measurements of electrophysiological parameters (see below) were taken at 10 weeks of age.

Group “early control (EC)” were 6 ZDF fa/+ animals, and group “early diabetic (ED)” were 10 ZDF fa/fa animals undergoing left sciatic nerve block
immediately after baseline testing at 10 weeks. One week later, behavioral and electrophysiologic measurements were repeated, and the left sciatic nerve was excised for neurohistopathologic evaluation.

The group “late control (LC)” consisted of 10 fa/+ animals kept until 18 weeks of age. The group of diabetic animals for the late experiments were randomized into one of two groups according to a pre-existing randomization list: group “late diabetic without insulin (LD)” were 10 ZDF fa/fa diabetic animals kept until 18 weeks of age. Group “late diabetic with insulin (LDI)” were 10 ZDF fa/fa animals, which received 1½ subcutaneous insulin implants (LinPlant, LHR-10BV, LinShin, Toronto, Canada) using a custom-made trocar G12-SS, LinShin) at 10 weeks of age. The latter were dosed according to weight at 10 weeks, approximately 300g, and released approximately 3U insulin per day for a period of 60 days, covering our experimental period. Groups LC, LD, and LDI underwent a second, “late baseline” electrophysiologic testing at 18 weeks of age, followed by sciatic nerve block. One week later, behavior and electrophysiology tests were repeated, and tissue excision for neurohistopathology was performed.

Serum glucose levels were measured in all experimental groups in blood drawn from the left tail vein, using a commercially available glucose meter (Blue, FIA Biomed, Emsdetten, Germany), regularly calibrated using the LI and LII calibration solutions (FIA Biomed). In groups EC and ED, this was done at 10 and 11 weeks, and in groups LC, LD, and LDI, this was done at 10, 14, 16, and 18 weeks of age.

Electrophysiology

With temperature maintained well above 34°C using a warming blanket (HK25, Beurer, Ulm, Germany), we studied the sciatic and caudal nerve with monopolar needle electrodes as described previously using a Nicolet Viking IVP electromyography system (Nicolet, Madison, WI). In brief, for motor conduction studies of the sciatic nerve the recording cathode was placed in the intrinsic muscles between the hallux and the second digit, and the recording anode was placed subcutaneously on the lateral surface of the fifth digit. Stimulating electrodes were inserted 3 mm apart at the medial ankle, and just cranial to the sciatic notch. A
grounding electrode was attached between the stimulating and the recording electrodes. Supramaximal square-wave pulses of 0.1 ms duration were delivered. Supramaximal stimulation was achieved by increasing the intensity by 25-30% above the maximal stimulation. Compound muscle action potential (CMAP) amplitudes (peak to peak) were recorded. Motor nerve conduction velocity (MNCV) was calculated over the segment between the sciatic notch and the ankle, and minimal F-wave latency was measured from seven F-wave recordings.

For studies of the mixed sensory and motor caudal nerve, the recording cathode and anode were inserted at the base of the tail just 5 mm apart. The stimulating cathode was placed laterally in the tail at exactly 3 cm from the base of the tail; the stimulating anode was inserted 5 mm distal to the stimulating cathode. The earth electrode was attached halfway between the stimulating and recording electrodes. Supramaximal square-wave pulses of 0.5 ms duration were delivered. Compound nerve action potential (CNAP) amplitudes (negative peak) were recorded. The nerve conduction velocity (NCV) of the tail nerve was calculated from the latency of the stimulus artefact to the onset of the negative peak of the action potential elicited and the distance between the stimulating and the recording cathodes, which was a standard distance of 3 cm. All measurements were carried out by one investigator (P.L.), and electrophysiological measurements underwent blinded assessment and validation by an experienced neurophysiologist (C.V.).

**Sciatic nerve block**

Nerve block was performed percutaneously combining the technique described by Thalhammer et al. modified by nerve stimulation as described by Kroin et al. In brief, a 25G needle was introduced just caudal to the sciatic notch directed cephalad, and connected with a clip to the Viking electromyography system programmed to deliver a pulse of 0.1 ms duration, and 0.6 mA current, triggered manually. Ipsilateral hind-leg kick in the absence of local stimulation was taken as sign of proximity of needle to nerve, and injection of 0.2 mL of lidocaine 2% was performed. We defined a successful nerve block on the basis of three signs:

1) before injection, successful nerve stimulation at 0.6 mA current,
2) gradual disappearance of the CMAP in electrophysiological recordings after injection of lidocaine, and
3) subsequent behavioural testing showing absence of the toe-spreading reflex.

The latter reflex, used to test sciatic nerve fibres, was tested as described before by Kroin et al. Animals were gently lifted, resulting in a physiologic vestibular reflex where toes are extended and spread. We noted the presence or absence of these findings to characterize block duration every 15 minutes until the block subsided. This gross behavioural testing was repeated before the animals were subjected to anaesthesia one week after nerve block to detect any permanent nerve injury.

**Neurohistopathology**

The main outcome parameter was the nerve injury score of the left sciatic nerve one week after nerve block. To this end, after the last electrophysiological measurements and animal euthanasia, left sciatic nerves were excised from animals. The segment proximal and distal from the site of injection was harvested, and fixed with a 2% buffered formalin solution, embedded in paraffin, cut at six microns in longitudinal and transverse sections and stained with hematoxylin-eosin (H.E.) and Masson trichrome. Samples were examined under the light microscope for evidence of inflammation in the epineurial, perineurial and endoneurial compartment, vascular injury and nerve fibre injury. Nerve fibre injury was assessed employing a simple, semi-quantitative four point score where 0 represents a normal nerve and 4 represents extreme injury with inflammation and destruction of all components of the nerve including axons and myelin, extending throughout the nerve bundle. The pathologist (U. de G.) was blinded to experimental group allocation.

**Statistical analysis**

Semiquantitative neurohistopathological data such as the primary outcome were compared by Friedman test followed - if significant - by Mann-Whitney U test. Power analysis revealed that a group size of 10 animals would have 80% power to reject the null hypothesis that the neurohistopathology score in the one group is
significantly different from another group of nerves using Mann-Whitney U test with a 0.05 two-sided significance level. Body weight, blood glucose level and neurophysiologic data were compared by analysis of variance (ANOVA) between groups followed by posthoc Bonferroni test for multiple comparisons. Variations of neurophysiological data over time were compared by paired samples T test. P < 0.05 was considered significant. Statistical analysis was performed with IBM SPSS® Statistics Version 20 (IBM, San Francisco, CA). Power analysis was done with the aid of nQuery Advisor® 7.0 (Statistical Solutions Ltd., Cork, Ireland).

Results

All animals survived to the end of the experiment, no animal needed analgesic rescue, and no animal fulfilled predefined criteria for termination of experiments (humane endpoints). Welfare assessment showed no abnormalities concerning appearance or behaviour at any time point. All animals showed clinical recovery from sciatic nerve block.

Early diabetes

At baseline (10 weeks), mean glucose value was 7.1 ± 1.0 in EC and 13.1 ± 4.9 mmol/l in ED animals (P < 0.01). Mean body weight was 288.8 ± 8.5 respectively 344.1 ± 16.7 g (P < 0.001). In EC versus ED nerves, sciatic nerve MNCV was 39.3 ± 4.2 versus 35.7 ± 2.5 m/s at baseline (P = 0.02), minimal F-wave latency was 7.3 ± 0.5 ms versus 7.9 ± 0.6 (P < 0.005).

Sciatic nerve block duration was 45 ± 13 min in the EC, and 67.5 ± 27 min in the ED group (P = 0.08).

The differences between electrophysiological parameters at baseline and 1 week after sciatic nerve block were calculated. Mean MNCV was decreased 1 week after block compared to baseline across EC and ED animals (P < 0.01). There was no difference between EC and ED animals in their change over time. We found no significant differences in electrophysiological CMAP parameters for controlled and EC animals at baseline and after nerve block.

In histopathological investigations most specimens in the EC and ED group showed mild chronic inflammation in the epineurium and in the adipose tissue. In
the EC group, 1/6 animals had a minimally elevated nerve injury score of “1+” (out of 4), compared to 3/10 animals in the ED group with an elevated nerve injury score of “1+” (n=1) and “2+” (n=2, n.s.).

Late diabetes

Weight and glucose levels of test animals are given in Table 1. Diabetic animals were randomized at 10 weeks of age to receive insulin treatment (LDI) or no treatment (LD). At 14 weeks of age, the mean glucose values were significantly lower in LDI than in LD animals. However, thereafter the difference became not significant (Table 1). Neurophysiologic data of conduction velocity and minimal F-wave latency are given in Figure 2. Conduction velocity increased over time in the LC group whereas it remained unaltered in LD and LDI animals. The conduction velocity at 18 weeks in the LD animals was significantly lower than in the LC group and remained significantly different one week after sciatic nerve block. After nerve block, conduction velocity tended to decrease in all groups, but this decrease did neither reach statistical significance in any of the separate groups nor when data of all groups were pooled (p = 0.15, Figure 2A). We found no significant differences in electrophysiological CMAP parameters for LC and LD animals before and after nerve block. Caudal NCV was slowed significantly when comparing animals in groups LC (65.3 ± 12 m/s) and LD (57.1 ± 8 m/s, P < 0.05).

At 18 weeks, minimal F-wave latencies were significantly prolonged in the LD as compared to the LC group. At one week after nerve block the latency increased significantly, if data of all three groups (LC, LD, LDI) were pooled (Figure 2B). Decreases in minimal F-wave latency between the three experimental groups were not significant.

Block duration was shortest in the LC group, longest in the LD group, and intermediate in the LDI group (Figure 2C). The LDI animals had a longer block than the LC animals, but there was no significant difference in block duration between LD and LDI animals. LD animals had a block duration significantly longer (94.5 ± 33.2 min) than ED animals (67.5 ± 27 min, P < 0.05).

In histopathological investigations, we noted only minor changes. LC animals showed minimal, variable, multi-focal chronic inflammatory infiltrates in
epineural connective tissue, generally not extending into the endoneurial or perineurial compartments. One animal out of the LDI group and one animal out of the LD group showed focal oedema or focal area of acute myelin injury and axonal damage, associated with scattered inflammatory cells. The neurohistopathological changes were not significant between groups (Figure 3).

Discussion

Zucker Diabetic Fatty rats at 10 weeks of age did not show prolongation of duration of sciatic block with lidocaine 2% and did not show a discernible impact on nerve damage one week after sciatic nerve block. In rats 18 weeks of age, with more longstanding diabetes and clear signs of neuropathy, sciatic nerve block duration was substantially prolonged, but no gross behavioural signs, or increased neurohistopathological signs of nerve injury were found one week after sciatic nerve block. Electrophysiologic changes suggestive of subtle nerve dysfunction after nerve block were present in all experimental groups, but this was not related to the duration or severity of the neuropathy.

The ZDF model used in the present study represents the important patient collective of Type II DM more accurately than the previously used STZ-induced Type I DM model. Notably, pathogenesis differs considerably between Type I and Type II diabetes in experimental models, and in humans, such that implications for regional anaesthesia should preferably be undertaken in a model most closely resembling the clinical situation. However, all previous investigations had been conducted in models of Type I DM in vivo, or in Type II DM in vitro. Our investigation is the first to investigate the effects of a DPN secondary to Type II DM on toxicology and function of sciatic nerve blockade.

Early diabetes

In our model, ED rats at 10 weeks of age had mildly decreased nerve conduction velocities, indicating a mild diabetic neuropathy. The neuropathy in these rats develops over time and our measurements of neuropathy correspond well with previous literature. Duration of nerve block was not significantly prolonged \((P = 0.08)\) in ED as compared to EC animals. We found no significant
neurohistopathologic or gross behavioural signs of nerve damage one week after sciatic nerve block. Our results concerning neurotoxicity correspond to previous *in vitro* investigations in the ZDF model at 12 weeks of age,\(^{11}\) and with similar findings in an *in vivo* model of Type I DM.\(^{23}\)

**Late diabetes**

LD animals at 18 weeks of age had decreased nerve conduction velocities as compared to LC animals, indicating the development of a more severe diabetic neuropathy over time. This corresponds to previous literature.\(^{25}\) We found that while there was a slight increase in MNCV in LC over time, the MNCV of LD did not show such an increase. This is in concordance with previous work by Oltman and colleagues, where a slight increase in MNCV in lean (healthy) animals over time until the 20th week can be observed.\(^{24}\) Also, we corroborated our MNCV findings by simultaneous measurements of the caudal NCV, confirming diabetic neuropathy also in this predominantly sensory nerve.

We found no histopathological signs of increased neurotoxicity in LD animals as compared to age-matched LC animals, when lidocaine 2% was used to elicit sciatic nerve block. Our results mirror previous investigations in type I DM, in which lidocaine at clinical concentrations had limited neurotoxic effects.\(^{10,21}\) There have been no *in vivo* local anaesthetic neurotoxicity investigations in type II diabetic models at all, but recent *in vitro* data show only modest neurotoxicity of lidocaine 2%.\(^{11}\) Our data confirm and widen these *in vitro* results using a multifaceted testing setup *in vivo*. Further, epidemiologic clinical outcome data suggest that even when long-lasting local anaesthetics are used, nerve injury following neuraxial blockade in patients with pre-existing neuropathy is rare. Specifically, in one retrospective study, the incidence of apparent neuropathic complications after neuraxial anaesthesia was 2 in 325 patients.\(^{6}\) Across the past 20 years, 6 case reports describing association of nerve damage with diabetic neuropathy following regional anaesthesia have been published.\(^{5}\)

We note highly significant prolongation of minimal F-wave latency as a subtle marker of nerve dysfunction one week after sciatic nerve block. This has not been previously described, but there was no difference between LD and LC animals,
Regional anesthesia in early and late neuropathy

such that this most likely represents a minor and unspecific sequel of nerve block. The clinical importance of this remains unclear, and is most probably very limited. There has been discussion whether regional anaesthesia in diabetic patients may induce clinically unapparent damage which may promote progression of diabetic neuropathy. However, the changes observed here are very small, and need to be investigated in detail before any clinical relevance can be ascribed.

In LD animals, block duration was significantly prolonged, while LDI animals had an intermediate increase in block duration. This finding was expected on basis of previous findings. Our results differ in magnitude from our previous manuscript, in which several tests were used to quantify motor, deep sensory, and superficial sensory block. In comparison, our rather crude “on/off” testing for a vestibular reflex in this study more closely reflects the data obtained by Kroin, who used the same method. The main difference with the latter study is that we used 0.2 mL of 2% lidocaine as in our previous study, whereas Kroin used 0.1 mL of 1% lidocaine. Several studies assessed block duration in a streptozotocin-induced rat model of type I DM, and while one study found no difference, three studies showed prolonged block duration in diabetic rats. This latter finding was confirmed in the ZDF rat model of type II DM by us. Recently, two clinical studies demonstrated increased sciatic nerve block duration in diabetic patients. Therefore, the evidence strongly indicates that diabetic neuropathy will prolong the duration of peripheral nerve block. This prolongation may be caused by pharmacokinetic or pharmacodynamic (e.g., modulation of sodium channels by neuropathy) mechanisms, but the respective contributions remain unclear. Potential clinical implications are to consider the diabetic nerve “more sensitive” to the effects of local anaesthetics, and it has been proposed to reduce dose of local anaesthetics when performing nerve blocks for perioperative analgesia. Also, it had been suggested to reduce or omit epinephrine from peripheral nerve blocks in neuropathic patients, and the results obtained in experimental and clinical settings would indicate that nerve block duration in diabetic neuropathy will be prolonged anyway, even without the need to add adjuvant epinephrine.
Limitations

In the LDI rats the effects of insulin were not sufficient to cause glucose levels to be similar to those in control animals, even though the same dose achieved good glucose control in type I \(^{23}\) and type II \(^{28}\) models of DM. This may be because insulin was dosed according to body weight at baseline (10 weeks), and diabetic animals were substantially heavier at the end of the experimental period, leading to relative under-dosing towards the end of the experimental period, which is supported by the increasing blood glucose levels over time in these animals. Therefore, our insulin regimen was more representative of loose glycaemic control than of strict glycaemic control.

In diabetic as well as control animals, small inflammatory changes were noted on neurohistopathological investigation in all groups, which may be explained by repeated stimulation. Sciatic nerve inflammation after repetitive stimulation, as occurred in our study due to neurophysiological measurements, has been described in vivo before.\(^{29}\) The timepoint of excision was chosen on basis of earlier experiments by our group, but differ with the timepoint chosen by Kroin et al.\(^{10}\) (2 days post block). Seen that the mild changes after block were seen both in the study by Kroin and our study, these results can be interpreted to support and strengthen each other.

The model used by us cannot be extrapolated directly to the clinical situation. First, the age of the experimental rat can be compared to that of a young human adult when estimating on basis of physiological and behavioural parameters.\(^{30}\) However, it should be noted that we chose the age of our test animals based on the age at which neuropathy typically develops, which is around 20 weeks.\(^{24}\) Second, the precise interspecies difference between rats and humans concerning neuropathy and toxicity of local anaesthetics is unclear. Nevertheless, rodent models have been used to investigate diabetes and its neuropathy,\(^{24}\) and determine functional and toxicological aspects of regional anaesthesia in the past.\(^{31}\)

Lastly, we attest to the fact that the focus of behavioural testing after sciatic nerve block was on the motor component of the nerve block, while DPN profoundly affects sensory function as well.\(^{1}\) However, in a previous study using the same
model, we obtained comparable prolongations of both motor and sensory blockade upon sciatic nerve blockade.\textsuperscript{11}

**Ethical considerations**

We sought to minimize animal distress by conducting all invasive procedures such as electrophysiology and nerve block under general anaesthesia. To limit tissue injury and prevent post-interventional pain, we performed nerve blocks percutaneously as described by Kroin et al.,\textsuperscript{10} and not open. At the same time, this theoretically entails that the position of injection is less reliable, and intraneural injection is possible. However, the only study directly comparing these two modes of injection found comparable results for both approaches,\textsuperscript{10} such that we felt confident to perform our blocks percutaneously. The same percutaneous approach using thin needle electrodes was chosen for electrophysiological measurements. To avoid repetitive injections of insulin for the animals randomised to the late group, we used subcutaneous implants. To minimize the number of animals used in experiments, we obtained approval to use the heart and the right sciatic nerve of our test animals for pilot experiments investigating enzyme expression and epigenetic markers in type II diabetes, respectively.

**Future perspectives**

We describe a novel comprehensive model to investigate toxicological and functional consequences of diabetic neuropathy *in vivo*, combining behavioural, electrophysiological and histopathology methods. Despite lidocaine being the most widely used local anaesthetic for toxicity research, results obtained by Kroin et al.\textsuperscript{10} suggest that longer-acting local anaesthetics such as bupivacaine and/or ropivacaine may be more toxic with respect to neurohistopathology.\textsuperscript{10} We suggest to investigate the neurotoxic potential of long-lasting local anaesthetics such as bupivacaine, and the value of adjuvants, which may increasingly become relevant in clinical practice.\textsuperscript{7} \textsuperscript{26} \textsuperscript{32} Similarly, the pharmacokinetics and –dynamics of the diabetic nerve as relevant to regional anaesthesia warrant further investigation.
Conclusions

Our results suggest increased sensitivity of diabetic nerves for short-acting local anaesthetics without adjuvants in vivo, as evidenced by prolonged block duration in a rodent Type II diabetes mellitus model with longstanding diabetic neuropathy. This sensitivity appears to increase with progression of neuropathy. We observed very subtle changes suggestive of nerve injury after nerve block in general, with no correlate in gross behavioural testing or neurohistopathology, and no specific effect of neuropathy. Our results do not support the hypothesis that neuropathy due to Type II diabetes mellitus increases the risk of nerve injury after peripheral nerve block.

Acknowledgments

We acknowledge the expert technical assistance of Marian Slaney BSc in preparing neurohistopathologic samples for analysis, of Shu-Hsien Sheu MD PhD for digitalization of neurohistopathologic specimens, and of Susanne van Dieren PhD for statistical advice.

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Regional anesthesia in early and late neuropathy


### Table 1

**Body weight (gr)**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>LC (n=10)</th>
<th>LD (n=10)</th>
<th>LDI (n=10)</th>
<th>LC vs. LD</th>
<th>LC vs. LDI</th>
<th>LD vs. LDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>282 ± 14</td>
<td>345 ± 19</td>
<td>350 ± 20</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>364 ± 17</td>
<td>419 ± 39</td>
<td>471 ± 25</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>386 ± 23</td>
<td>427 ± 38</td>
<td>484 ± 35</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>402 ± 37</td>
<td>435 ± 36</td>
<td>494 ± 41</td>
<td></td>
<td>n.s.</td>
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</tr>
</tbody>
</table>

**Blood glucose (mmol)**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>LC (n=10)</th>
<th>LD (n=10)</th>
<th>LDI (n=10)</th>
<th>LC vs. LD</th>
<th>LC vs. LDI</th>
<th>LD vs. LDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.6 ± 1.9</td>
<td>11.9 ± 5.3</td>
<td>11.5 ± 4.6</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.001</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>6.0 ± 0.5</td>
<td>24.2 ± 5.1</td>
<td>17.8 ± 7.4</td>
<td>n.s.</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>6.2 ± 0.5</td>
<td>25.5 ± 3.2</td>
<td>21.6 ± 5.0</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.001</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>8.1 ± 1.2</td>
<td>28.4 ± 3.4</td>
<td>25.2 ± 5.5</td>
<td></td>
<td>p &lt; 0.01</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Weight and blood glucose levels of all groups over time.
Tested by ANOVA with posthoc Bonferroni correction. n.s. = not significant
Figure legends

**Figure 1**
Timeline of experimental interventions.

**Figure 2**
Development of sensible conduction velocity (2A) and minimal F-wave latency (2B) over time in the three experimental groups. After nerve block, conduction velocity tended to decline in all groups, but did not reach statistical significance even if all groups were pooled (p = 0.15).
After the nerve block the minimal F-wave latency increased significantly, when the data of all three groups were pooled.
Analysis of variance (ANOVA) with posthoc Bonferroni test, paired samples T test. *P < 0.05; #P < 0.01; ##P < 0.001.

**Figure 3**
Sciatic nerve motor block duration in late diabetic animals. Analysis of variance (ANOVA) between groups followed by posthoc Bonferroni correction. *P < 0.05; #P < 0.01; ##P < 0.001.

**Figure 4**
Neurohistopathology of a healthy control nerve nerve (A), and a nerve from a diabetic animal, showing focal areas of acute myelin injury and axonal damage, associated with scattered chronic inflammatory cells (B).
Figure 1

Early Diabetes
Group Early control (EC)
Group Early diabetic (ED)

Electrophysiology
Sciatic nerve block

9 weeks old
10 weeks old
11 weeks old

Late Diabetes
Group Late control (LC)
Group Late diabetic (LD)
Group Late diabetic with insulin treatment (LDI)

Electrophysiology
Group LDI: Insulin implant

9 weeks old 10 weeks old
18 weeks old 19 weeks old

Electrophysiology
Sciatic nerve block

Electrophysiology
Neurohistopathology

//
Figure 2: Neurophysiology of tibial nerve

2A
Regional anesthesia in early and late neuropathy

2C

![Block duration diagram]

Block duration

- p < 0.01
- p < 0.05

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td></td>
</tr>
<tr>
<td>LDI</td>
<td></td>
</tr>
</tbody>
</table>

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Figure 3
Management of patients with diabetic neuropathy

Based on

Lirk P, Rutten MVH, Haller I, Stevens MF, Laudolff-Birmingham J, Hollmann MW, Birmingham B

Survey of the management of the patient with diabetic peripheral neuropathy presenting for regional anesthesia.

Introduction

Diabetes mellitus is one of the most common diseases worldwide, and its prevalence is predicted to increase tremendously within a decade. Among other end-organ complications, it may lead to diabetic peripheral neuropathy (DPN). It is estimated that about 10% of diabetic patients presented with neuropathy at the time of diagnosis, while more than 50% of diabetic patients will develop neuropathy during the subsequent years, making it the most common neuropathy worldwide.

The diagnosis of diabetic neuropathy may influence perioperative management, especially the decision whether or not to perform regional anaesthesia. Reasons to perform surgery under regional anaesthesia may include the wish to avoid general anaesthesia in light of comorbidities. Some types of surgery for diabetic complications are highly suitable for regional anaesthesia, e.g., arteriovenous fistula in end-stage renal disease. On the other hand, DPN may increase risk of neurological injury following regional anaesthesia. Recommendations have been issued regarding the performance of peripheral regional anaesthesia in patients with DPN arguing for, among others, decreased doses of local anaesthetics to decrease the chance of adverse outcome.

However, these recommendations rest on a very limited evidence-base. There is limited epidemiological evidence suggesting that DPN may predispose patients to new-onset neurological symptoms, and some case reports describe associations between adverse outcomes of regional anaesthesia and pre-existing neuropathy. Experimental evidence is equivocal, and there are studies to support and to refute increased toxicity of local anaesthetics in DPN. Recently, increased attention has been paid to the possibility that DPN may prolong nerve block duration.

It has not been determined whether these controversial results and recommendations have been incorporated into clinical practice. Therefore, this study first sought to assess reported practice in the perioperative management of patients with DPN presenting for peripheral regional anaesthesia among members of the European Society of Regional Anaesthesia and Pain Therapy (ESRA). Second, we wanted to contrast findings from our survey with a review of a current status of the literature concerning peripheral regional anaesthesia in diabetic neuropathic patients.
Management of the neuropathic patient

We hypothesized that the majority of responders would consider DPN a potential risk factor for nerve damage in peripheral regional anaesthesia, and would adapt their technique.

Materials and Methods

Survey

The survey was approved by the ESRA Scientific Board. We developed a web-based survey questionnaire in English using an online survey engine (www.surveymonkey.com), validated in a pilot study by two study authors (PL, MVR) and one independent anaesthetist. An anonymous e-mail containing an invitation to participate in the survey was distributed to members of ESRA by the society’s secretariat in June 2012, with an anonymous reminder email sent two weeks later, and the survey remained open for two months. To ensure confidentiality, survey responses did not contain personal information or data which would allow identification of an individual or his institution. No free text input was possible. The survey collected demographic data and assessed practitioners’ routine methods for preoperative assessment and intraoperative management of patients with suspected diabetic neuropathy presenting for regional anaesthesia. The complete survey questionnaire can be found in the Appendix. To increase the response rate, participants were given the opportunity to sign up for the drawing of three textbooks of anaesthesia following the survey. All personal information entered for this purpose was kept separate from survey answers at all times.

Literature review

For each section of the literature review ("local anaesthetic neurotoxicity", “regional anaesthesia adjuvants”, “nerve block duration” and “nerve stimulation”), we performed a comprehensive literature search for reports in journals indexed in MEDLINE covering manuscripts until November 2012, with reference lists of retrieved articles searched for additional trials or reports. The initial search strings were “diabetic neuropathy” and “regional anaesthesia”, and “neurotoxicity”.

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Statistics

All responses were stored on a secure database and were exported to IBM SPSS Statistics 20 (Armonk, NY) for analysis. The data collected in this survey were mostly categorical in nature. Demographic variables are strictly categorical, while the variables coded on the Likert scale were considered ordered. We first carried out single (uni-variate) analysis of the data collected for each variable. Subsequently, we constructed contingency tables to study association between answers related to our main hypothesis, and Pearson’s Chi-square test for independence was used to discover if there is a relationship between two categorical variables. To address the concern of small expected numbers of values in crosstabulation, some of the variables were recoded combining levels of the variable with low cell counts.

Results

Survey

A total of 3,343 emails were sent to members of the European Society of Regional Anaesthesia (ESRA), of which 236 could not be delivered, resulting in a final number of 3,107 emails delivered. We received 584 responses with fully completed questionnaires, which represented an overall response rate of 19%.

Demographics - We received responses from European anaesthesiologists (34 countries, 509 responders), while 69 participants were located outside of Europe and 6 participants gave no information. The countries with high numbers of responders were the United Kingdom (n = 164, 24 %), followed by France, Belgium, Netherlands and Italy, each one accounting for more than 5 % of the respondents. Anaesthesia training had been completed by 91% of responders. While more than 80% of responders stated the amount of regional anaesthesia performed at their institution was below 40 %, the personal practice of our responders seemed slightly above what was practiced at their institution. Demographic data are given in detail in Table 1.
Management of the neuropathic patient

**Block technique** - When asked about the main techniques used for nerve localization, ultrasound was preferred (51%), with double guidance (ultrasound, nerve stimulator; 41%) and nerve stimulator (30%) alone used less frequently. Other techniques were used very seldom (Table 2A). Anaesthesiologists with more years of experience tended to use nerve stimulator techniques more often and they were less likely to use ultrasound. The same trend was observed for anaesthesiologists from institutions with a higher utilization of peripheral regional anaesthesia. Similarly, anaesthesiologists at smaller hospitals tended to use nerve stimulator more often, and dual guidance less often than their colleagues at larger hospitals.

**Neuropathy** - When asked about the approach to the diabetic patient, a minority of participants would order a neurologic consult and the same holds true for patients with confirmed diabetic neuropathy (Table 2B). Anaesthesiologists from smaller hospitals, with more years of experience or who performed more peripheral regional anaesthesia were more likely to consult a neurologist in case of diabetes and/or neuropathy. In neuropathic patients 17 % of participants stated they would be inclined to avoid peripheral nerve blocks, yet 59 % of participants would counsel patients about possibly increased risk of nerve damage.

In patients with confirmed neuropathy, approximately a quarter of participants would modify the technique of regional anaesthesia, a third would do so sometimes, and while 41 % rarely or never do so (Table 2C). When technique was to be altered, 43 % of responders stated they would usually or always decrease the dose of local anaesthetic, almost 77 % would regularly decrease or omit epinephrine, and 40 % would accept a higher nerve stimulation threshold (Table 2D).

**Personal valuation** - Figure 1 summarizes the respondents’ agreement or disagreement with to statements regarding the risk of peripheral regional anaesthesia in patients with diabetic neuropathy. Answers were not associated with years of experience or site of practice. Anaesthesiologists who agreed that performing peripheral nerve blocks on patients with diabetic peripheral neuropathy increases their risk of postoperative neurologic deficit (Figure 1A), and those who agreed that regional anesthesia in DPN patients
would increase medical liability risk (Figure 1B) were more attentive to duration and severity of diabetes and diabetic neuropathy, and more likely to inform the patient about increased risk concerning peripheral regional anaesthesia. Moreover, they were more likely to alter their technique or avoid regional anesthesia at all. Anaesthesiologists who agreed to the statement that general anaesthesia is, in principle, superior to regional anaesthesia in diabetic neuropathic patients (Figure 1C) tended to use a nerve stimulator alone, and were less likely to use ultrasound. In addition to being more attentive to symptoms of diabetes and DPN, these colleagues were more inclined to order neurology consults preoperatively, change technique, or avoid nerve blocks altogether.

Anaesthesiologists who agreed with the statement that peripheral nerve blocks can be performed safely in patients with diabetic neuropathy (Figure 1D) tended to use ultrasound alone, and not nerve stimulation. They were also less likely to order a neurologic consult for patients with diabetes or diabetic peripheral neuropathy. In addition they were less inclined to counsel their patients of an increased risk of nerve damage, and they did not alter their technique of peripheral nerve blocks.

Regional practice differences – The only geographical sub-group of responders lending itself to in-depth analysis was responders from the United Kingdom, representing 24% of all responses. In comparison to responses from colleagues from continental Europe, UK anaesthetists tended to use less regional anaesthesia in their daily practice, and tended to use the nerve stimulator technique less often. They more often inquired about the symptoms of peripheral neuropathy but were less likely to order neurologic consults. They also less likely to modify their technique than their counterparts in Continental Europe were, and more likely to avoid peripheral nerve blocks in neuropathic patients altogether. They were more likely to counsel the patient about the increased risk of regional anaesthesia.

Future research - Finally, 82% of responders agreed with the statement that research regarding the safety of peripheral nerve blocks in patients with diabetic peripheral neuropathy should be given high priority in the future.
Literature search

Results of the literature search are summarized in Tables 3 and 4. No randomized controlled trial examining potential adverse effects of peripheral regional anaesthesia in diabetic neuropathy was found. We retrieved one epidemiological study, one prospective clinical trial examining spinal block duration in diabetic patients, two clinical trials examining sciatic nerve block duration, one retrospective investigation examining block success in diabetic patients, and 6 single case reports (Table 3). Moreover, we found 7 experimental studies in three different animal models of diabetes (Table 4).

Discussion

The results from our literature search reveal inconsistent findings concerning a potential increase in local anaesthetic-induced neurotoxicity in diabetic patients, but there is consensus that diabetic neuropathy will prolong block duration (Table 2). This view is mirrored in the survey. Few responders are inclined to request a neurologic consult in patients with confirmed neuropathy prior to performing peripheral regional anaesthesia, about a quarter of participants stated they would avoid regional anaesthesia in patients with neuropathy, yet almost 60% would regularly counsel patients with neuropathy about increased risk of nerve damage as a consequence of regional anaesthesia. When techniques are modified, most participants decrease or omit epinephrine, while fewer respondents would decrease dose of local anaesthetic or perform other adjustments.

In summarizing responses, two large groups became obvious: one smaller group of regional anaesthetists who are more cautious concerning diabetic neuropathy, more likely to use a nerve stimulator only, and who are inclined to use general anaesthesia in diabetic neuropathic patients. The second, larger, group is characterized by the predominant use of ultrasound, and a more optimistic approach, less likely to order consults, and of the opinion that nerve blocks can safely be performed in diabetic neuropathic patients. Stratification according to years of experience or site of practice does not explain this difference in attitude. In analyzing the subgroup of UK anaesthetists, a more conservative approach was observed, with a higher likelihood of avoiding regional anaesthesia in neuropathic...
patients, and increased chance of counseling neuropathic patients about perceived greater risks of regional anaesthesia.

**Local anaesthetic neurotoxicity**

The respondents were divided regarding the risk of new-onset neurologic deficit in neuropathic patients. Concerning risk of regional anaesthesia in these patients, strong agreement or disagreement was very rare. This reflects the scarce data support in this field. Guidelines issued by the American Society of Regional Anesthesia (ASRA) pay attention to patients with pre-existing neurological disease, such as DPN. With regards to local anaesthetic neurotoxicity, “consideration may be given” to avoiding more potent local anaesthetics, and reducing the dose or concentration of local anaesthetic.\(^5\)

The clinical evidence that diabetic neuropathy may be a risk factor for nerve damage rests, to a large extent, on case reports and case report series summarized in Table 3. The only respective epidemiological study was conducted on diabetic neuropathic patients undergoing neuraxial anaesthesia. Hebl and coworkers retrospectively analyzed the incidence of new-onset neuropathy, and found an incidence of 0.4\(^\%\).\(^6\) In contrast with other recent estimates of risk,\(^17\) this seems substantially increased, but the number of complications observed was very small and therefore, extrapolation is not straightforward.\(^3\)

Clinical evidence is underpinned by equivocal experimental evidence. In a first landmark study, Kalichman and Calcutt demonstrated in streptozotocin diabetic rats (as a model of type I diabetes) that lidocaine was more toxic in diabetic nerves.\(^13\) Using the same model, Kroin and coworkers found only small increases in neurotoxic effects when long-lasting local anaesthetics, or adjuvants such as epinephrine and clonidine were used.\(^14\) Results obtained in dissociated neurons of Zucker Diabetic Fatty (ZDF) rats (as a model of type II diabetes) similarly show only a marginal increase in toxicity when exposed to lidocaine in vitro.\(^16\) Other evidence was obtained in a spinal injection model in type I diabetic rats. Even though these results cannot be directly extrapolated to peripheral regional anaesthesia, it is notable that no increased pathologic findings were observed when animals were subjected to 4 subsequent injections of high-dose lidocaine (2\(^\%\)) or
bupivacaine (0.75%) with or without epinephrine. In conclusion, experimental results are conflicting. One observed trend is that higher concentrations of local anaesthetic are more likely to cause neurotoxic effects in diabetic animals (see Table 4). However, results from animal studies, including very specific models such as streptozotocin-induced DM, can only be extrapolated to the clinical situation with great care.

Based on the available evidence, it has been suggested to lower the dose of local anaesthetic in the presence of severe DPN. In diabetic animals, recovery of nerve function from anoxia or ischemia-reperfusion was significantly delayed as compared to healthy control animals, suggesting that diabetic nerves are, in general, more sensitive to toxic challenges than are healthy nerves (Table 4). Further research should include late diabetic neuropathic patients and compare the influence of early versus late neuropathy on the toxicological safety profile of local anaesthetics. It is notable that despite the uncertainty in contemporary literature, approximately sixty percent of responders stated to counsel neuropathic patients about the potentially increased risk of nerve damage with regional anaesthesia. Many colleagues perform clinical exams to determine preoperative status in patients with DPN. From a legal / forensic point of view, this is highly recommended. It should be noted, however, that many new-onset neurologic symptoms following regional anaesthesia are due to factors unrelated to the blockade, such as positioning.

Regional anaesthesia adjuvants

Among those respondents who would change nerve block technique in patients with confirmed DPN, almost eighty percent would decrease or omit epinephrine as adjuvant to local anaesthetics. This coincides with several recommendations to decrease or omit epinephrine in diabetic neuropathic patients. Whether epinephrine is acceptable in the context of a test dose remains to be determined, and the theoretical risk of increased neurotoxicity needs to be weighed against the danger of an unrecognized intravascular injection with potential deleterious effects in its own right. As mentioned above, limited evidence suggests epinephrine may increase toxic effects of lidocaine and other local anaesthetics.
because nerve blood flow is impaired, and the concentration of local anaesthetic remains elevated for longer periods.\textsuperscript{14}

\textit{Nerve block duration}

While the first study by Kalichman\textsuperscript{13} did not show a prolongation of block duration, subsequent studies by others\textsuperscript{14 15 19} and us\textsuperscript{16} demonstrate increased block duration in diabetic versus non-diabetic animals. Recent reports in patients support the concept of longer block duration in diabetic patients. Specifically, Sertoz investigated diabetic patients undergoing popliteal sciatic nerve block stratified into three groups according to their glycosylated hemoglobin levels, a surrogate marker of recent glucose regulation. Notably, block onset was delayed, and duration prolonged in patients with the highest glycosylated levels.\textsuperscript{20} Next, Cuvillon and coworkers showed that in patients with diagnosed DPN, ropivacaine-induced subgluteal sciatic nerve block onset time was similar, but sensory and motor block duration was prolonged by four hours, respectively.\textsuperscript{20} In line with these clinical reports, a recent study in diabetic rats showed that acute glycemic control did not reverse the duration of anaesthesia to normal levels, indicating that long-term changes rather than the current blood glucose level are most likely responsible for this phenomenon.\textsuperscript{19} The reason for increased sensitivity may be pharmacodynamic (altered pattern of ion channel composition or modification in diabetic neurons\textsuperscript{21}), or pharmacokinetic (decreased absorption due to angiopathy\textsuperscript{22}). Moreover, a recent study comparing pregnant diabetics versus non-diabetics receiving lidocaine epidural anaesthesia revealed decreased clearance of lidocaine and its metabolite monoethylglycinexylidine (MEGX), indicating that diabetes as such may impair the function of cytochrome P450 enzymes responsible for local anaesthetic breakdown.\textsuperscript{23} Finally, a recent retrospective study found a higher block success rate in diabetic patients undergoing surgery under supraclavicular block, potentially because nerves are already partially numb due to neuropathy, or more sensitive toward effects of local anaesthetics.\textsuperscript{24}
**Nerve stimulation**

We found that a relatively large number of responders use nerve stimulation, and almost 40% of responders stated they were inclined to accept a higher stimulation threshold in neuropathic nerves. A quarter of participants stated that they would tend to avoid the use of a nerve stimulator in diabetic patients. It seems reasonable that a nerve which has undergone degenerative changes and does not conduct normal stimuli properly might not be optimally suited for nerve stimulator guided regional anaesthesia. Experimental evidence in a small number of diabetic dogs showed that the possibility of intraneural injection was increased in diabetic versus healthy dogs. Recently, using ultrasound combined with nerve stimulation, Bigeleisen and colleagues demonstrated that stimulation thresholds were increased in diabetic patients. Severe diabetic neuropathy may be an indication to prolong the stimulating pulse or employ double guidance (simultaneous use of ultrasound and nerve stimulation).

**Limitations**

Some limitations of the present study should briefly be addressed. First, the response rate was low. However, considering the open and non-personal manner in which society members were recruited into the survey we feel that the response rate was as expected. To maximize response rate, we drafted an invitation letter explaining the aim and potential importance of the study, devised a survey that could be completed in less than five minutes, sent a reminder two weeks later, and invited participants to sign up for a prize draw of three major textbooks of Anaesthesia. In addition, we attest to the fact that the survey population was a selected group of colleagues, with membership in a dedicated specialist society. Because almost three quarters of responders perform > 20% of regional anaesthesia in daily practice, we surmise that the survey was completed by a group of anaesthesiologists with a dedicated interest in regional anaesthesia. Our survey was designed in English, which may have constituted a language barrier for some recipients of the invitational email, precluding them from answering. Preliminary analysis of regional differences showed that participants from the United Kingdom had a more conservative and cautious stance when dealing with diabetic neuropathic patients. No data are...
available on the approach to the diabetic neuropathic patient in other world regions. Future surveys focused on types of hospitals and different regions will be required to delineate differences in attitudes and practices in more detail. Moreover, it may be necessary to differentiate between various forms of neuropathy, specifically concerning onset, treatment, and localization. Finally, our survey did not take into account the distinctive pathophysiology of neuropathy during diabetes mellitus Type I (primarily due to insulin shortage) and Type II (due to insulin resistance). Whereas glucose control is very crucial in the neuropathy of Type I diabetes, the disease aetiology is more complex in Type II diabetes. In the latter form, other factors such as dyslipidemia contribute to the pathogenesis of DPN. Future clinical and experimental studies will need to take these factors into consideration.

Conclusions

In conclusion, we report the results of the first survey analyzing attitudes and standards of care among European anaesthesiologists with regards to peripheral regional anaesthesia in Diabetic Peripheral Neuropathy. While literature is divided on the question whether pre-existing diabetic neuropathy is a risk factor for new neurological deficit after regional anaesthesia, many of the respondents take measures to reduce risks, counsel patients on a perceived greater risk of neurologic complications, and only a minority avoids peripheral regional anaesthesia altogether.

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220
Management of the neuropathic patient

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6 Hebl JR, Horlocker TT, Schroeder DR. Neuraxial anesthesia and analgesia in patients with preexisting central nervous system disorders. *Anesth Analg* 2006; **103**: 223-8, table of contents


9 Horlocker TT, O'Driscoll SW, Dinapoli RP. Recurring brachial plexus neuropathy in a diabetic patient after shoulder surgery and continuous interscalene block. *Anesth Analg* 2000; **91**: 688-90


11 Blumenthal S, Borgeat A, Maurer K, et al. Preexisting subclinical neuropathy as a risk factor for nerve injury after continuous ropivacaine administration through a femoral nerve catheter. *Anesthesiology* 2006; **105**: 1053-6

12 Angadi DS, Garde A. Subclinical neuropathy in diabetic patients: a risk factor for bilateral lower limb neurological deficit following spinal anesthesia? *J Anesth* 2012; **26**: 107-10


Chapter 3.5


20 Sertoz N, Deniz MN, Ayanoglu HO. Relationship between glycosylated hemoglobin level and sciatic nerve block performance in diabetic patients. Foot Ankle Int 2013; 34: 85-90


Table 1 - Demographics of survey responders

<table>
<thead>
<tr>
<th>Primary site of practice</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community hospital</td>
<td>53 %</td>
</tr>
<tr>
<td>University hospital</td>
<td>44 %</td>
</tr>
<tr>
<td>Outpatient surgery center</td>
<td>1 %</td>
</tr>
<tr>
<td>Office based anesthesia</td>
<td>2 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size of hospital / practice</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 250 beds</td>
<td>20 %</td>
</tr>
<tr>
<td>251 – 500 beds</td>
<td>28 %</td>
</tr>
<tr>
<td>501 – 750 beds</td>
<td>20 %</td>
</tr>
<tr>
<td>751 – 1000 beds</td>
<td>17 %</td>
</tr>
<tr>
<td>&gt; 1000 beds</td>
<td>15 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anesthesia experience</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>In training</td>
<td>9 %</td>
</tr>
<tr>
<td>0 – 5 years</td>
<td>20 %</td>
</tr>
<tr>
<td>5 – 10 years</td>
<td>24 %</td>
</tr>
<tr>
<td>10 – 20 years</td>
<td>29 %</td>
</tr>
<tr>
<td>&gt; 20 years</td>
<td>18 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regional anesthesia in institution and own practice</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 20 %</td>
<td>43 %</td>
</tr>
<tr>
<td>20 – 40 %</td>
<td>38 %</td>
</tr>
<tr>
<td>40 – 60 %</td>
<td>13 %</td>
</tr>
<tr>
<td>60 – 80 %</td>
<td>4 %</td>
</tr>
<tr>
<td>80 – 100 %</td>
<td>2 %</td>
</tr>
</tbody>
</table>
Table 2 - Survey responses regarding Block technique and Neuropathy

A.

<table>
<thead>
<tr>
<th>Localization aid employed</th>
<th>always/usually</th>
<th>sometimes</th>
<th>seldom/never</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve stimulator alone</td>
<td>30 %</td>
<td>25 %</td>
<td>45 %</td>
</tr>
<tr>
<td>Ultrasound alone</td>
<td>51 %</td>
<td>23 %</td>
<td>26 %</td>
</tr>
<tr>
<td>Dual guidance</td>
<td>41 %</td>
<td>26 %</td>
<td>33 %</td>
</tr>
<tr>
<td>Others</td>
<td>3 %</td>
<td>19 %</td>
<td>78 %</td>
</tr>
</tbody>
</table>

B.

<table>
<thead>
<tr>
<th>In diabetics, assess …</th>
<th>always/usually</th>
<th>sometimes</th>
<th>seldom/never</th>
</tr>
</thead>
<tbody>
<tr>
<td>… duration of diabetes</td>
<td>82 %</td>
<td>10 %</td>
<td>8 %</td>
</tr>
<tr>
<td>… symptoms of neuropathy</td>
<td>51 %</td>
<td>23 %</td>
<td>26 %</td>
</tr>
<tr>
<td>… neurologic assessment</td>
<td>41 %</td>
<td>26 %</td>
<td>33 %</td>
</tr>
<tr>
<td>… neurologic consultation</td>
<td>3 %</td>
<td>19 %</td>
<td>78 %</td>
</tr>
</tbody>
</table>

C.

<table>
<thead>
<tr>
<th>In neuropathics …</th>
<th>always/usually</th>
<th>sometimes</th>
<th>seldom/never</th>
</tr>
</thead>
<tbody>
<tr>
<td>… request neuro consult</td>
<td>12 %</td>
<td>17 %</td>
<td>71 %</td>
</tr>
<tr>
<td>… avoid nerve blocks</td>
<td>17 %</td>
<td>37 %</td>
<td>46 %</td>
</tr>
<tr>
<td>… counsel about ↑ risk</td>
<td>59 %</td>
<td>19 %</td>
<td>22 %</td>
</tr>
<tr>
<td>… modify technique</td>
<td>28 %</td>
<td>31 %</td>
<td>41 %</td>
</tr>
</tbody>
</table>

D.

<table>
<thead>
<tr>
<th>Modifying block by …</th>
<th>always/usually</th>
<th>sometimes</th>
<th>seldom/never</th>
</tr>
</thead>
<tbody>
<tr>
<td>… ↓ local anesthetic dose</td>
<td>43 %</td>
<td>24 %</td>
<td>33 %</td>
</tr>
<tr>
<td>… ↓ epinephrine</td>
<td>77 %</td>
<td>9 %</td>
<td>14 %</td>
</tr>
<tr>
<td>… ↓ other adjuvants</td>
<td>59 %</td>
<td>15 %</td>
<td>26 %</td>
</tr>
<tr>
<td>… avoid nerve stimulator</td>
<td>25 %</td>
<td>20 %</td>
<td>55 %</td>
</tr>
<tr>
<td>… accept higher st. thr.</td>
<td>40 %</td>
<td>25 %</td>
<td>35 %</td>
</tr>
</tbody>
</table>

st. thr. stimulation threshold
Table 3 - Clinical studies or case reports reporting comparisons among patients with or without DPN.

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>N</th>
<th>Block</th>
<th>LA</th>
<th>Tox</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidemiological</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hebl 2006&lt;sup&gt;6&lt;/sup&gt;</td>
<td>R</td>
<td>567</td>
<td>Neuraxial</td>
<td>Bupi</td>
<td>+</td>
</tr>
<tr>
<td>Case reports</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waters 1996&lt;sup&gt;7&lt;/sup&gt;</td>
<td>R</td>
<td>1</td>
<td>2 x Spinal</td>
<td>Tetracaine 1%</td>
<td>+</td>
</tr>
<tr>
<td>Lena 1998&lt;sup&gt;8&lt;/sup&gt;</td>
<td>R</td>
<td>1</td>
<td>Epidural</td>
<td>Lido 2%</td>
<td>+</td>
</tr>
<tr>
<td>Horlocker 2000&lt;sup&gt;9&lt;/sup&gt;</td>
<td>R</td>
<td>1</td>
<td>Interscal</td>
<td>Bupi 0.16%</td>
<td>+</td>
</tr>
<tr>
<td>Al-Nasser 2004&lt;sup&gt;10&lt;/sup&gt;</td>
<td>R</td>
<td>1</td>
<td>Epidural</td>
<td>Ropi 0.75%</td>
<td>+</td>
</tr>
<tr>
<td>Blumenthal 2006&lt;sup&gt;11&lt;/sup&gt;</td>
<td>R</td>
<td>1</td>
<td>Spinal / Femoral</td>
<td>Ropi</td>
<td>+</td>
</tr>
<tr>
<td>Angadi 2012&lt;sup&gt;12&lt;/sup&gt;</td>
<td>R</td>
<td>1</td>
<td>Spinal</td>
<td>Bupi 0.5%</td>
<td>+</td>
</tr>
<tr>
<td><strong>Clinical trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echevarria 2008&lt;sup&gt;13&lt;/sup&gt;</td>
<td>P</td>
<td>88</td>
<td>Spinal</td>
<td>Bupi 0.5%</td>
<td>x</td>
</tr>
<tr>
<td>Gebhard 2009&lt;sup&gt;14&lt;/sup&gt;</td>
<td>R</td>
<td>262</td>
<td>Supraclav</td>
<td>Bupi 0.5%</td>
<td>x</td>
</tr>
<tr>
<td>Sertoz 2013&lt;sup&gt;15&lt;/sup&gt;</td>
<td>P</td>
<td>45</td>
<td>Popliteal</td>
<td>Levo 0.5%</td>
<td>x</td>
</tr>
<tr>
<td>Cuvillon 2013&lt;sup&gt;16&lt;/sup&gt;</td>
<td>P</td>
<td>72</td>
<td>Sciatic</td>
<td>Ropi 0.475%</td>
<td>x</td>
</tr>
</tbody>
</table>

R retrospective P prospective. Tox Toxicity + increased x not reported. O Final patient outcome + resolution - persistent deficits x not reported D duration increased. S success rate increased.
Table 4 - Experimental studies reporting block duration and neurologic symptoms in test animals with or without DPN.

<table>
<thead>
<tr>
<th>Author / Year</th>
<th>Animal</th>
<th>DM</th>
<th>Block</th>
<th>LA</th>
<th>Tox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalichman 1992</td>
<td>rat</td>
<td>STZ I</td>
<td>sciatic</td>
<td>Lidocaine 2%</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lidocaine 4%</td>
<td>++</td>
</tr>
<tr>
<td>Lindstrom 1994</td>
<td>rat</td>
<td>BB II</td>
<td>sciatic</td>
<td>Anoxia</td>
<td>x</td>
</tr>
<tr>
<td>Baba 2006</td>
<td>rat</td>
<td>STZ I</td>
<td>sciatic</td>
<td>Ischemia</td>
<td>+</td>
</tr>
<tr>
<td>Kroin 2010</td>
<td>rat</td>
<td>STZ I</td>
<td>sciatic</td>
<td>Lidocaine 1%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lido / Epin</td>
<td>≈</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lido / Clonid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ropivacaine</td>
<td>+</td>
</tr>
<tr>
<td>Kroin 2012</td>
<td>rat</td>
<td>STZ I</td>
<td>spinal</td>
<td>Lidocaine 1%</td>
<td>o</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lido / Epin</td>
<td>o</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bupivacaine</td>
<td>o</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bupi / Epin</td>
<td>o</td>
</tr>
<tr>
<td>Kroin 2012</td>
<td>rat</td>
<td>STZ I</td>
<td>sciatic</td>
<td>Lido / Epin</td>
<td>x</td>
</tr>
<tr>
<td>Lirk 2012</td>
<td>rat</td>
<td>ZDF II</td>
<td>sciatic</td>
<td>Lido 2%</td>
<td>o</td>
</tr>
</tbody>
</table>

DM diabetes mellitus. LA local anesthetic. /epin with added epinephrine. /clonid with added clonidine. STZ streptozotocin rat model. BB biobreeding Worcester rat model. ZDF Zucker Diabetic Fatty rat model. + increased - decreased ≈ similar o no difference x not performed/reported.
Figure 1 – Personal valuation of Neuropathy.

Legend ++ agree strongly, + agree somewhat, o neutral, - disagree somewhat, -- disagree strongly.
Section 4

*Epigenetic effects of local anesthetics*
Chapter 4.1

*Lidocaine demethylates DNA in breast cancer cells in vitro*

Based on

Lirk P, Berger R, Hollmann MW, Fiegl H

*In vitro, lidocaine demethylates DNA in breast cancer cells*

*Br J Anaesth* 2012; 109: 200-207
Introduction

Surgical tumour removal remains a highly relevant treatment option for cancer patients, despite concerns that the perioperative period may facilitate progression of the underlying disease due to immunosuppression, surgical stress, and administration of drugs suspected of promoting tumour spread. Regional anaesthetic procedures, or the intravenous administration of local anaesthetics, have both been demonstrated to reduce perioperative surgical stress. The ultimate clinical relevance of these effects is unclear, and currently available evidence is undecided as to whether potential positive effects of regional anaesthesia are relevant enough to influence patient outcome after cancer surgery. Current large-scale multicenter randomized controlled trials are projected to last until the end of the decade.

In the meantime, research is focused on potential mechanisms of local anaesthetic-induced tumour suppression. Several pathways have been described in literature. On one hand, local anaesthetics, at high doses, are cytotoxic in vitro, and on the other hand, they may induce sensitization of tumour cells to chemotherapeutics and heat. Another potential mechanism whereby local anaesthetics may influence tumour growth is by interaction with the tumour epigenome. In malignancy, increased methylation frequently leads to down-regulation of tumour suppressor genes, favouring tumour progression.

The prototype ester-type local anaesthetic, procaine, has been shown to demethylate DNA and inhibit tumour growth and in the MCF-7 breast cancer cell line, and similar results were later obtained in hepatoma and leukaemia cell lines. Epigenetic effects of amide-type local anaesthetics, such as lidocaine, have not been examined. Given the structural differences between these two types of local anaesthetics, differential effects on epigenetic features are possible. In addition, lidocaine is very widely used in contemporary regional anesthesia, and increasingly employed intravenously in the context of multimodal treatment regimens.

We sought to examine the effects of the prototype amide-type local anaesthetic, lidocaine, on DNA methylation in prototypical breast cancer cell line
Lidocaine demethylates DNA in breast cancer cells

cultures. Our working hypothesis was that exposure of cancer cells to lidocaine would decrease DNA methylation.

Methods

Cell culture

Human breast cancer cell lines BT-20 (estrogen receptor-negative) and MCF-7 (estrogen receptor-positive) were obtained from the American Type Culture Collection (ATCC) and were cultured according to company recommendations. Amplification of 15 short tandem repeat (STR) loci and the gender-specific locus amelogenin was carried out in the Institute of Legal Medicine of the Medical University Innsbruck to authenticate the cell lines, using 10 ng of template DNA applying the Geneprint PowerPlex 16 System (Promega) following the manufacturer’s recommendations as previously described.

Drug treatments

The following drugs were purchased from Sigma Aldrich (Vienna, Austria): lidocaine n-ethyl bromide (L5783) and procaine hydrochloride, (P9879), both dissolved in distilled water. We treated BT-20 and MCF-7 breast cancer cell lines with a final varying concentration lidocaine, procaine and the positive control substance, 5 µM 5-aza-2′-deoxycytidine (DAC) for 72 and 96 hours respectively. Twenty-four hours after seeding, the medium was removed and replaced with medium containing the drug solutions at the desired final concentration. DAC was dissolved in DMSO to a final concentration of 10 mM, aliquoted, and stored at -20°C. Lidocaine and procaine were dissolved in water to a final concentration of 1 M and 0.5 M respectively, aliquoted, and stored at -20°C. Whenever needed, a fresh aliquot was diluted to the desired final concentration.

Effect of lidocaine and procaine on cell proliferation.

We analyzed the effects of lidocaine and procaine on cell proliferation in the human breast cancer cell lines BT-20 and MCF-7 during 72 hours and 96 hours incubation by counting the cell number. To this end, Breast cancer cells were seeded into medium size cell culture bottles in MEM medium with 10% FCS (MCF-7: 2.2
Mio cells, BT-20: 1 Mio cells) and treated 24 hours later. After the indicated incubation time, 72 hours or 96 hours respectively, the cells were trypsinized and counted (Beckman coulter). Biotin-labeled POD TUNEL Apoptosis detection kit for adherent cell was used according to manufacturer’s protocol (GenScript, Piscataway, NJ). Cells were visualized using an Olympus 1X70 inverted Microscope in conjunction with Kappa ImageBase software V2.7.2 (Gleichen, Germany).

**Global Genomic DNA Hypermethylation**

Global genomic 5-methylcytosine content was determined by quantitative MethyLight assay specific for Chromosome 1 SAT2 repeat sequences. We analyzed the effects of 1mM procaine, different lidocaine concentrations (0.01mM, 0.1mM, 1mM) and 5µM DAC on the global DNA methylation status in MCF-7 and BT-20 breast cancer cells after 72, 96 and 120 hours (Fig. 2). Genomic DNA from treated cells was extracted using the DNeasy tissue kid (Qiagen) method. Sodium bisulfite conversion of genomic DNA and MethyLight was performed as described previously.

**DNA methylation and RNA expression of several Tumour Suppressor Genes**

We decided to test the effects of lidocaine in comparison to procaine on particular hypermethylated loci in both cell lines. RASSF1A, GSTP1 and MYOD1 are known epigenetically inactivated genes in breast cancer. Primers and Probes for COL2A1 (reference gene), RASSF1A, MYOD1 and GSTP1, and Chromosome 1 juxta-centromeric satellite 2 (SAT2) DNA sequences have recently been published. We compared the effects of 1mM lidocaine and procaine. Total cellular RNA was extracted by the standard acid guanidium thiocyanate-phenol-chloroform method and reverse transcription of RNA was performed as previously described. Primers and probe for Real time quantitative PCR analysis (qPCR) for RASSF1A, MYOD1 and GSTP1 were purchased from Applied Biosystems (AB Assay ID: Hs00200394_m1, Hs00159528_m1 and Hs00168310_m1). Primers and probes for the TATA box-binding protein (TBP; a component of the DNA-binding protein complex TFIIID; was used as endogenous RNA control) were used according to Bieche et al. Real-time PCR was performed using an ABI Prism 7900HT.
Detection System (Applied Biosystems, Foster City, CA). The standard curves were generated using serially diluted solutions of standard cDNA derived from the Hs578T carcinoma cell-line.

Statistics

Results are expressed as mean ± standard deviation (SD). The unpaired Student's t-test was used for the comparison of the various effects after the different treatments in normally distributed data, and the Mann-Whitney U test for non-normally distributed data. P-values less than 0.05 were considered as statistically significant. SPSS 17.0 was used for the statistical analyses.

Results

Effect of lidocaine and procaine on cell proliferation.

Treatment with 1 mM procaine or 1 mM lidocaine resulted in significant reductions in cell number, while lower concentrations of local anaesthetics did not lead to significant cell number reduction (Fig. 1A). In both cell lines no increase in the apoptosis rate was observed upon lidocaine (1 mM, 0.1 mM, 0.01 mM) or procaine (1 mM) treatment respectively (Fig. 1B).

Global Genomic DNA Hypermethylation

At baseline, methylation was 100-fold higher in BT-20 than in MCF-7 cells. At equal dose, lidocaine was a stronger demethylating agent than procaine (Fig. 2). In MCF-7 cells, we observed demethylation after 96, but not 72, hours after treatment with 1 mM procaine (Fig. 2A; p=0.004). Treatment with 1 mM lidocaine resulted in a statistically significant increase of global Sat2 DNA methylation after 72 hours, whereas the treatment with 0.1 mM and 0.01 mM lidocaine revealed a significant demethylation after 72 and 96 hours (Fig. 2A; p<0.001, respectively). In comparison to procaine, treatment with 0.1 mM or 0.01 mM lidocaine revealed a stronger demethylating effect (Fig. 2A). Even in comparison to the demethylating agent, DAC, the treatment with 0.1 or 0.01 mM lidocaine revealed a more significant decrease of DNA methylation (Fig. 2A).
In BT-20 cells, the treatment with 1mM procaine revealed a significant decrease of DNA methylation after 72 and 96 hours (Fig. 2B; p<0.001 or p=0.019, respectively). We observed a dose-dependent decrease in DNA methylation in response to lidocaine (1mM, 0.01mM, 0.01 mM) after 72 hours (Fig 2B; p<0.001, p<0.001 or p=0.004, respectively) and 96 hours (Fig. 2B; p<0.001, p=0.034 or p=0.38, respectively), similar to effects noted upon incubation with DAC.

Additionally we treated the breast cancer cells with 0.01mM, the clinically relevant dose of lidocaine, for 48, 72, 96 and 120 hours. We observed a statistical significant demethylating effect in MCF-7 after 72 to 120 hours, whereas in BT-20 cells the demethylating effect was seen only between 72 and 96 hours (Fig. 3).

DNA methylation and RNA expression of specific Tumour Suppressor Genes

In MCF-7 cells, after 72 hours or 96 hours respectively, neither DNA-methylation nor mRNA expression changes in our three exemplary tumour suppressor genes were observed (Fig. 4). Only for \textit{MYOD1} we observed a statistical significant increase in DNA methylation after 72 hours in lidocaine treated MCF-7 cells (Fig. 4A; p=0.006), without effecting the \textit{MYOD1} mRNA expression. In procaine treated cells we identified an increase in \textit{MYOD1} mRNA expression after 72 hours (Fig. 4B; p=0.036). DAC as control substance decreased methylation of the three tumour suppressor genes (Fig. 4A), and resulted in an increased mRNA expression only for \textit{MYOD1} after 72 hours (Fig. 4B).

In BT-20 cells we identified no decrease in methylation or mRNA expression upon procaine or lidocaine treatment after 72 or 96 hours. The treatment with DAC gave rise to a decreased \textit{RASSF1A}, \textit{GSTP1} and \textit{MYOD1} DNA methylation, and increased the mRNA expression of these three tumour suppressor genes (Fig 4B).
Lidocaine demethylates DNA in breast cancer cells

Discussion

The present study demonstrates that lidocaine time- and dose-dependently demethylates DNA in BT-20 breast cancer cells (estrogen receptor-negative cell line), whereas in MCF-7 cells (estrogen-receptor-positive cell line) this demethylating effect was only observed to a smaller, albeit statistically significant, extent. This effect was noted at concentrations corresponding to those reached after systemic application of local anaesthetics, or after systemic absorption from epidural or paravertebral anaesthesia. These concentrations are, however, insufficient to cause direct cytotoxicity. Elicitation of cytotoxic effects would necessitate concentrations of local anesthetic that can only be reached after direct application of local anesthetic on the tumour intraoperatively. Finally, epigenetic modification appears to be transient, since demethylation was returned to non-significant levels after 120 hours in BT-20 cells. While global methylation status was profoundly influenced, the expression status of three exemplary tumour suppressor genes (RASSF1A, GSTP1 and MYOD1) was not significantly changed, such that further studies will need to elucidate which genes are affected by the demethylating effects of lidocaine.

The potential clinical implication of these results is that a potent pathway leading to malignancy seems to be influenced by local anaesthetics at clinically relevant doses. This may, in part, explain beneficial effects of regional anaesthesia on cancer recurrence observed in some studies. Furthermore, the magnitude of this effect is dependent upon epigenetic features of the tumour type. Whereas we observed statistically significant alterations in methylation status in both exemplary cell lines, effects were much more pronounced in an ER-negative cell line, which is associated with high methylation levels at baseline. Thus, beneficial effects relating to epigenetic modulation of cancer biology may be limited to certain types of tumours. The ultimate clinical relevance of our findings remains to be determined.

The issue whether the mode of anaesthesia influences outcome after cancer surgery has been a topic of intense debate. In a retrospective study on melanoma patients, Schlagenhauff et al. found that patients who had undergone melanoma excision under general anaesthesia had a decreased survival as compared to those
receiving local (infiltration) anaesthesia only. It should be noted that anaesthetic infiltration of peri-neoplastic tissues leads to very high regional concentrations of local anaesthetic in the milimolar range. These concentrations of local anaesthetics are known to be cytotoxic when applied to tumour cell lines. In contrast, when local anaesthetics are administered during epidural or paravertebral anaesthesia, low micromolar concentrations are attained in the systemic circulation. In breast cancer patients, a retrospective study revealed a substantial benefit with regards to metastatic spread in patients in whom paravertebral anaesthesia had been used during mastectomy. Corresponding data on abdominal cancer patients could, however, not replicate these promising findings, except for a subgroup of older patients in one study. In another study, all-cause mortality was reported to be reduced after resection of rectal, but not colonic, malignancies when epidural anaesthesia was part of anaesthetic technique. Results regarding prostate cancer patients remain equivocal. Two studies in patients suffering from ovarian carcinoma have linked intraoperative epidural analgesia to increased 3- and 5-year survival, and increased recurrence-free interval, while epidural anesthesia during brachytherapy had no beneficial survival effect in patients with cervical cancer. In patients undergoing percutaneous radiofrequency ablation of hepatocellular carcinoma, use of epidural anesthesia was associated with decreased survival. All these equivocal results may indicate that either, regional anaesthesia has no effect, or, they may be explained by the biological heterogeneity of tumours. Our results indicate that lidocaine influences cell lines with different biological properties differently, exerting strong demethylating effects on ER-negative, and less pronounced effects on ER-positive, cell lines. Thus, the question of whether regional anaesthesia or, for that sake, other components of the perioperative care process, modulate cancer recurrence is perhaps not one that can be answered unequivocally. Rather, our findings would indicate that tumour-suppressive effects of local anaesthetics may only detectable in specific types of cancer.

The role of local anaesthetics in tumour progression may be indirect or direct. Indirect actions of regional anaesthesia include attenuation of the neuroendocrine response to surgery followed by improved preservation of
immunocompetence, and effects on the administration of drugs suspected of modulating tumour growth, e.g., opioid-sparing effect. Direct actions are caused by direct effects of local anaesthetic on tumour cells, or sensitizing effects towards other therapeutic measures such as thermo- and chemotherapy. Local anaesthetics have been shown to protect against tumour cell invasion in concentrations attained clinically after local infiltration and suppress the proliferation of a number of tumour cell lines. Our results suggest that next to classic cytotoxic effects seen at doses exceeding those attained clinically, local anaesthetics are also able to subtly modulate tumour biology. Modulation of tumour epigenome was observed at far lower doses than those needed to elicit overt cytotoxicity. Cancer treatment strategies based upon epigenetics are being developed, and the ester-type local anaesthetic, procaine, had been suggested as a candidate substance. Our results suggest that lidocaine is an even more potent demethylating agent than procaine.

The stability and expression of the human genome is controlled by methylation of specific regions of desoxyribonucleic acid (DNA). Next to the classic four nucleotides adenine, thymine, guanine, and cytosine, a fifth base, 5-methylcytosine, confers epigenetic features to control a variety of physiologic and pathologic processes. Increasing evidence suggests epigenetic aberrations as a major pathogenic factor in the development of malignancy. Modulation of tumour suppressor or promoter genes has been described as a key event in cancer. In short, increased methylation can lead to silencing of tumour suppressor genes, thereby promoting tumour progression. In these cases, decreased methylation may be of therapeutic benefit. Catalyzation of methylation is achieved by the family of DNA methyltransferases (DNMT). DNMT1 seems to be the main pathogenic enzyme in human cancer, while its suppression can significantly inhibit tumour growth. We hypothesized that similar to procaine, lidocaine leads to demethylation by inhibiting DNMT. In an early landmark study, Kennedy et al. postulated that sensitizing effects of lidocaine for the chemotherapeutic, bleomycine, were potentially caused by interference with DNA integrity. We show that in vitro, lidocaine is an even more potent DNA-demethylating agent than procaine.
The differential effect of lidocaine at a concentration of 1mM in BT-20 and MCF-7 cell lines is a novel finding, but not unexpected given the heterogeneity of human cancer types. In part, it confirms previous ex vivo experiments, in which inhibitory effects of serum taken from patients undergoing regional anaesthesia impeded ER-negative cells in vitro. For example, breast cancer, which we chose to serve as the tumour paradigm for this study, has been subdivided into at least five distinct subtypes based upon gene expression profiling, and immunohistochemical markers. These are characterized by differential sensitivity to treatment, and clinical outcome, and biological heterogeneity is only beginning to be fully understood. In our case, BT-20 cells showed a much higher index of methylation than MCF-7 cells, with PMR values 100-fold higher in BT-20 cells (Fig. 2). Demethylating effects of local anaesthetics may depend on a high level of pre-existing methylation to show effect. Conversely, in cancer types in which DNA methylation is not a key pathogenic factor, the potential therapeutic effect seems negligible.

Finally, some potential limitations of our study should be briefly mentioned. First, we used lidocaine as a prototypical amide-type local anaesthetic, whereas in clinical routine, long-acting local anaesthetics are more frequently used for epidural anaesthesia and analgesia. We chose to investigate lidocaine as the first prototypical substance since it constitutes the most widely investigated local anaesthetic, and is the only local anaesthetic that is routinely administered locally for infiltration, for epidural or paravertebral anaesthesia, or intravenously as part of multimodal anaesthetic and analgesic regimens. Extrapolating from the fact that both procaine and lidocaine exert demethylating effects, the same should be anticipated for long-acting compounds such as bupivacaine and ropivacaine, even if testing this hypothesis was beyond the scope of the present study. These results were obtained in two cell lines representative of estrogen-positive and estrogen-negative breast cancer, respectively. These cell lines have been extensively researched and validated. Yet, they may feature genotypic and phenotypic drift over time. We sought to minimize this potential bias, by authenticating cell lines using amplification of several STR loci. Local anaesthetics have been demonstrated to
Lidocaine demethylates DNA in breast cancer cells

sensitize tumour cells towards cytotoxic effects of chemotherapeutics, and it is thus possible that such sensitization may also take place towards demethylating effects of novel chemotherapeutics.

Our findings suggest that demethylating tumour-suppressive effects of anaesthetic interventions may only be detectable in specific types of cancer due to differential methylation profiles. Lidocaine dose-dependently demethylates DNA of breast cancer cell lines in vitro.

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Figure Legends

Figure 1: Effect of lidocaine and procaine on cell proliferation of MCF-7 and BT-20 human breast cancer cells. (A) Cell numbers after lidocaine and procaine treatment. Results of 8 independent experiments are shown. (B) TUNEL assay. Results of 1 representative experiment out of 4 independent experiments are shown. Results are expressed as mean ±SD. Statistical significance between control and treated samples was assessed by the unpaired Student’s t-test (*, p < 0.05; ***, p < 0.001).

Figure 2: Global Genomic SAT2 DNA methylation analysis. (A) SAT2 DNA methylation levels in MCF-7 and (B) BT-20 breast cancer cells treated with 1 mM procaine, 1mM lidocaine, 0.1mM lidocaine, 0.01mM lidocaine or 5 μM 5-aza-2’-deoxycytidine (DAC) respectively. Results of 8 independent experiments are shown. Results are expressed as mean ±SD. Statistical significance between control and treated samples was assessed by the unpaired Student’s t-test (*, p < 0.05; ***, p < 0.001).

Figure 3: Global Genomic SAT2 DNA methylation analysis: time series. (A) SAT2 DNA methylation levels in MCF-7 and (B) BT-20 breast cancer cells treated with 0.01mM lidocaine for 48, 72, 96 and 120 hours. Results are expressed as mean ±SD. Results of at least 3 independent experiments are shown. Statistical significance between control and treated samples was assessed by the unpaired Student’s t-test (*, p < 0.05; ***, p < 0.001).

Figure 4: DNA methylation and RNA expression analysis of Tumour Suppressor Genes. (A) DNA methylation (results of 8 independent experiments are shown) and (B) mRNA expression (results of 5 independent experiments are shown) of RASSF1A, GSTP1 and MYOD1 after a treatment with 1mM procaine, 1mM lidocaine or 5µM DAC for 72 or 96 hours respectively. Results are expressed as mean ±SD. Statistical significance between control and treated samples was assessed
by the unpaired Student’s t-test or Mann-Whitney U test (for GSTP1 and MYOD1 mRNA expression) (*, p < 0.05; ***, p < 0.001).
Figure 1

Lidocaine demethylates DNA in breast cancer cells

Figure 1
Figure 2

A

MCF-7

B

BT-20

SATZ DNA methylation (PMR)

72 h procaine 1 mM
72 h lidocaine 1 mM
72 h lidocaine 0.1 mM
72 h DAC 5 µM
72 h control
96 h procaine 1 mM
96 h lidocaine 0.1 mM
96 h DAC 5 µM
96 h control

SATZ DNA methylation (PMR)

72 h procaine 1 mM
72 h lidocaine 1 mM
72 h lidocaine 0.1 mM
72 h DAC 5 µM
72 h control
96 h procaine 1 mM
96 h lidocaine 0.1 mM
96 h DAC 5 µM
96 h control
Lidocaine demethylates DNA in breast cancer cells

Figure 3
Figure 4
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*Lidocaine and ropivacaine, but not bupivacaine, demethylate deoxyribonucleic acid in cancer cells in vitro*

Based on

*Lirk P, Hollmann MW, Hauck-Weber NC, Fleischer M, Fiegl H*

*Lidocaine and ropivacaine, but not bupivacaine, demethylate deoxyribonucleic acid in cancer cells in vitro*

## Introduction

More than 100 years ago, it was recognized that tumour surgery makes metastasis more likely.\(^1\) The perioperative period of tumour surgery may well be a crucial time when patient outcome can be decisively influenced.\(^2\) Numerous perioperative factors which may explain tumour progression have now been identified. For example, resecting a primary tumour may promote the progression of distant metastases, or even induce tumour self-seeding, where circulating tumour cells re-infiltrate the original site of a resected tumour.\(^3\) This concept is supported by recent evidence that the number of circulating tumour cells increases dramatically in the perioperative period.\(^4\) One very important determinant of metastatic potential is the epigenetic signature of tumour cells.\(^5\)

In a healthy body, epigenetic mechanisms are responsible for the stability and expression of human deoxyribonucleic acid (DNA) as they regulate the methylation of specific DNA regions.\(^6\) Epigenetic mechanisms are increasingly recognized as pathogenic factors in several forms of cancer.\(^6\) Specifically, increases in methylation levels can deactivate tumour suppressor genes and lead to the progression of cancer.\(^6\), \(^7\) In these cases, decreasing methylation levels may be of therapeutic benefit. So a new class of chemotherapeutics designed to demethylate tumour DNA has been introduced into clinical practice.\(^8\)

We have recently shown that the prototype local anaesthetic, lignocaine, can reduce methylation of breast cancer cells at clinically relevant concentrations \textit{in vitro}.\(^9\) This study sought to determine whether similar demethylating effects could also be observed for two prototype long-acting local anaesthetics typically used for perioperative neuraxial anaesthesia: bupivacaine and ropivacaine. In addition, local anaesthetics have been described as enhancing the tumoricidal effects of conventional chemotherapeutics,\(^10\) and we wanted to investigate whether local anaesthetics would also increase the demethylating effect of a prototypical demethylating chemotherapeutic, 5-aza-2'-deoxycytidine (DAC).

The aim of this study was therefore to test two hypotheses: 1) that the local anaesthetics lignocaine, bupivacaine, and ropivacaine decrease methylation levels in tumour cells, and 2) that local anaesthetics enhance the demethylating effects of DAC.
Methods

Cell culture

Human breast cancer cell lines BT-20 (estrogen receptor [ER]-negative) and MCF-7 (ER-positive) were obtained from the American Type Culture Collection (ATCC, Manassas, USA) and were cultured according to ATCC recommendations. Amplification of 15 short tandem repeat (STR) loci and the gender-specific locus amelogenin was carried out in the Institute of Legal Medicine of the Medical University Innsbruck, Austria, to authenticate the cell lines. This was done using 10 ng of template DNA, applying the GenePrint PowerPlex 16 System (Promega, Madison, USA) according to the Manufacturer’s recommendations, as previously described.11

Drug treatments

The following drugs were purchased from Sigma Aldrich (Vienna, Austria): lignocaine N-ethyl bromide (L5783), bupivacaine hydrochloride monohydrate (B5274) and ropivacaine hydrochloride monohydrate (R0283), all dissolved in distilled water. We treated BT-20 and MCF-7 breast cancer cell lines with these local anaesthetics first alone and then in combination with varying concentrations of DAC for 72 hours. The following concentrations were used: 10 µM and 100 µM lignocaine, 2 µM and 20 µM bupivacaine, 3 µM and 30 µM ropivacaine; 0.001 µM, 0.02 µM, 0.1 µM, 0.2 µM 0.5 µM and 1 µM DAC. Twenty-four hours after seeding, the medium was removed and replaced with medium containing the drug solutions at the desired final concentration. DAC was dissolved in DMSO to a final concentration of 10 mM, aliquoted, and stored at -20°C. Lignocaine, bupivacaine and ropivacaine were dissolved in water to a final concentration of 1 M and 100 mM respectively, aliquoted, and stored at -20°C. Whenever needed, a fresh aliquot was diluted to the desired final concentration.

Effect of local anaesthetics on cell viability.

We analysed the effects of lignocaine in combination with DAC and bupivacaine and ropivacaine on cell viability in the human breast cancer cell lines BT-20 and MCF-7 during 72 hours’ incubation by the colorimetric MTT assay
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(M5655; Sigma, Vienna, Austria). The tetrazolium dye MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) was dissolved in RPMI-1640 without phenol red. The assay was performed according to Manufacturers’ instructions. Absorbance of converted dye was measured at a wavelength of 570 nm with background subtraction at 630–690 nm.\(^\text{12}\)

**Global Genomic DNA Hypermethylation**

Global genomic 5-methylcytosine content was determined by quantitative MethyLight assay, specific for Chromosome 1 SAT2 repeat sequences.\(^\text{13}\) We analysed the effects of 10 µM and 100 µM lignocaine, 2 µM and 20 µM bupivacaine and 3 µM and 30 µM ropivacaine alone or in combination with 0.001 µM, 0.02 µM, 0.1 µM, 0.2 µM, 0.5 µM or 1 µM DAC respectively on the global DNA methylation status in BT-20 and MCF-7 breast cancer cells after 72 hours. Genomic DNA from treated cells was extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany). Sodium bisulfite conversion of genomic DNA and MethyLight was performed as described previously.\(^\text{14}\)

**Additivity**

We sought to determine whether the interaction between DAC and lignocaine as the prototype local anaesthetic was supra-additive. Our calculations were based on the Loewe isobolographic additivity model, which has been described as particularly useful when investigating the interplay between two toxic substances in vitro.\(^\text{15}\) The half maximal DNA demethylation concentration (EC50) was calculated in BT-20 cells for DAC and lignocaine from at least 7 independent experiments at different concentrations of DAC and lignocaine, resulting in a preliminary EC50 of 0.08 µM for DAC, and 77.3 µM for lignocaine (line of additivity, Figure 4). We assumed supra-additive effects if 50% of demethylation were achieved with a combination of concentrations significantly lower than those representing the line of additivity.

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Statistics

Results are expressed as mean ± standard deviation (SD). The Mann-Whitney U test was used for the comparison of the various effects after the different treatments. *P*-values less than 0.05 were considered statistically significant. SPSS 17.0 (IBM, Vienna, The Austria) was used for statistical analyses.

Results

Effect of lignocaine, bupivacaine and ropivacaine on cell viability

Treatment with 10 µM or 100 µM lignocaine alone had no cytotoxic effect. Lignocaine at concentrations of 10 µM and 100 µM did not increase cytotoxicity of DAC in either BT-20 (Fig. 1A) or MCF-7 (Fig. 1B) breast cancer cell lines. Similarly, treatment with bupivacaine or ropivacaine at doses equipotent to lignocaine showed no cytotoxic effect in either breast cancer cell line (Fig. 1C, 1D).

Effect of bupivacaine and ropivacaine on global genomic DNA-methylation

Treatment with bupivacaine at 2 µM and 20 µM revealed no significant demethylating effect on global genomic DNA methylation in either breast cancer cell line BT-20 (Fig. 2A) or MCF-7 (Fig. 2B).

Treatment with ropivacaine for 72 hours at concentrations of 3 µM or 30 µM decreased methylation in BT-20 cells (Fig. 2A; *P*=0.003 or *P*=0.023, respectively). In MCF-7 cells, no demethylation was observed (Fig. 2B).

Effect of lignocaine, bupivacaine and ropivacaine in combination with DAC on global genomic DNA-methylation.

To determine whether local anaesthetics could increase the demethylating effect of DAC, we treated BT-20 and MCF-7 breast cancer cells for 72 hours with 10 µM and 100 µM lignocaine, 2 µM bupivacaine and 3 µM ropivacaine, and combined these treatments with DAC at several concentrations. We observed increased demethylation after the combined treatment of BT-20 cells with 0.1 µM DAC and 10 µM or 100 µM lignocaine respectively (Fig. 3A; *P*=0.014 or *P*=0.001 respectively) and 0.5 µM DAC with 100 µM lignocaine (Fig. 3A; *P*=0.008), in comparison to the DAC treatment alone. In MCF-7 cells, only the
combined treatment with 0.5 µM DAC and 10 µM lignocaine revealed a stronger demethylation in comparison to the mono-treatment with 0.5 µM DAC alone (Fig. 3B; \( P=0.006 \)). All other combined treatments of bupivacaine and ropivacaine with various concentrations of DAC revealed no increased demethylating effect in BT-20 or MCF-7 cells, as compared to treatment with DAC alone (Fig. 3).

**Additivity**

Since lignocaine was the most potent local anaesthetic agent as regards demethylating properties in this study, additivity experiments were based upon calculated EC50 in methylation for lignocaine and DAC. These theoretical results were compared to demethylation using several concentrations of DAC (0.02, 0.04, 0.08, 0.16, and 0.32 µM), combined with lignocaine (19.3, 38.7, 77.3, 154.6, 309.2 µM, respectively, Table 1). We then compared demethylation levels with the theoretical line of additivity (Figure 4), and found no supra-additivity (Figure 4, Table 1).

**Discussion**

This study was designed to test the hypotheses that 1) the local anaesthetics lignocaine, bupivacaine, and ropivacaine can decrease methylation levels in tumour cells, and that 2) lignocaine as the strongest demethylating agent would enhance the demethylating effects of DAC – the prototype epigenetic chemotherapeutic.

We found that 1) lignocaine and ropivacaine, but not bupivacaine, induce DNA demethylation, and that 2) lignocaine showed no supra-additive effects when combined with DAC.

The concentrations of local anaesthetics employed in the present investigation are in the range of concentrations reached during epidural infusion of local anaesthetics.\(^{16, 17}\) Equipotent concentrations of lignocaine, ropivacaine, and bupivacaine were calculated based on a previous electrophysiological study.\(^{18}\) Comparable doses of lignocaine (i.e. up to 10 µM) are observed after typical regimens of perioperative intravenous lignocaine infusion.\(^{19}\)
Epigenetic effects of local anaesthetics

Our results build upon and confirm previous results which indicated that at clinically relevant doses, lignocaine demethylates DNA in breast cancer cells in vitro.\textsuperscript{9} In addition, our results from individual drug treatments (Fig. 3), and Loewe additivity experiments (Fig. 4) suggest that the methylating effects of lignocaine and DAC are additive. We did not find evidence of supra-additivity. The mechanism by which local anaesthetics may influence methylation was not directly investigated in the present study, but procaine has previously been shown to inhibit DNA methyltransferase-I, the driving force behind the methylation of cytosine.\textsuperscript{20}

While local anaesthetics have been shown to make conventional chemotherapeutics work better in selected experimental settings,\textsuperscript{10, 21} no-one has yet tested their effects on the performance of demethylating chemotherapeutics. Further investigations are needed to determine the biological consequences of systemic lignocaine on tumour progression in the perioperative setting. In contrast to lignocaine, bupivacaine and ropivacaine seem less potent in inducing demethylation in tumour cells. The reasons for this differential effect can only be speculated upon. The equipotent doses chosen were based on sodium channel blockade,\textsuperscript{22} and so we surmise that the demethylating effect of these substances is not related to sodium channel blockade. This is not surprising since at least three alternative effects of local anaesthetics are mediated by pathways independent of sodium channel blockade. Firstly, different local anaesthetics show differential effects on G-protein-mediated priming of human neutrophils \textit{ex vivo}, which are not correlated with their potency in blocking sodium channels.\textsuperscript{23} Secondly, given equipotent doses, lignocaine is much more effective than bupivacaine in preventing thrombus formation.\textsuperscript{24, 25} And thirdly, Piegeler et al. demonstrated that the effects of lidocaine and ropivacaine on the phosphorylation of Src, a key molecule conjectured in tumour metastasis, were not related to potency at the sodium channel, and that ester-type local anaesthetics had no effect.\textsuperscript{26} In contrast to the latter study on Src phosphorylation however, the epigenetic effects of local anaesthetics are discernible in both amide-type and ester-type compounds. In a landmark manuscript, Villar-Garea showed that the prototype ester-type local anaesthetic, procaine, demethylated DNA and inhibited tumour growth in MCF-7 breast cancer cells,\textsuperscript{27} and others found
similar effects when investigating solid organ and haematopoietic tumour cell lines.  

Relevance of perioperative epigenetic modulation

The most important epigenetic alterations are methylation of cytosine residues in DNA to produce 5-methyl-cytosine, resulting in a change in the spatial configuration of histones. Most frequently, the pathologic epigenetic changes in malignancy involve increased methylation, which leads to the silencing of tumour suppressor genes. Modifications in the epigenetic signature of tumour cells are nowadays considered as important as genetic mutations themselves. In addition to this increasingly appreciated role in primary oncogenesis, epigenetic alterations are also increasingly understood to influence the prognosis and response to treatment of malignancy, including the probability of metastasis, in many tumours. Current treatments targeting the epigenome are associated with considerable side-effects. For example, the paradigmatic anti-epigenetic drug, decitabine (DAC), a potent inhibitor of DNA methylation, is associated with significant adverse effects such as myelosuppression and organ toxicity. We have previously shown that lignocaine, given at clinically relevant concentrations, acts as a demethylating agent. Here, we show that lignocaine shows additive demethylating effects when combined with DAC. Given the very good safety profile of systemic or regional administration of lignocaine, this drug offers potentially beneficial epigenetic effects in the perioperative period of tumour surgery.

Limitations

In larger concentrations, local anaesthetics can have direct cytotoxic effects on tumour cells, and this may explain protective effects against tumour recurrence observed after local anaesthesia for local superficial tumour excision. However, the concentrations used in the present study were found insufficient to cause direct cytotoxicity (Figure 1). Also, we note that the two tumour cell lines that we used have different baseline methylation properties. The effects of demethylation are largest in BT-20 cells, which have a high baseline methylation level. In the same way, biological heterogeneity may explain why specific anaesthetic interventions
Demethylating properties of lidocaine, bupivacaine and ropivacaine

seem to affect outcome in some types of cancer \(^{32,33}\) while no effect was found in other studies,\(^ {34}\) and some studies found effects only in defined subpopulations.\(^ {35}\)

Conclusions

Assessing our study alongside previous evidence, we conclude that, at clinically relevant doses, lignocaine and ropivacaine exert demethylating effects on breast cancer cells in vitro, but bupivacaine does not. When combined, lignocaine and DAC exhibit additive demethylating effects.

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Table 1: Global Genomic SAT2 DNA methylation in BT-20 breast cancer cell line after treatment with various concentrations of DAC, Lignocaine and a combined treatment.
DNA-methylation is indicated as percentage of a fully methylated reference (PMR). Data are presented as mean ± SD.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DAC [µM]</th>
<th>Lignocaine [µM]</th>
<th>SAT2 PMR value (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>after DAC treatment alone</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30 (± 7)</td>
</tr>
<tr>
<td>0.02</td>
<td>19.3</td>
<td>18 (± 5)</td>
<td>23 (± 1)</td>
</tr>
<tr>
<td>0.04</td>
<td>38.7</td>
<td>16 (± 4)</td>
<td>38 (± 7)</td>
</tr>
<tr>
<td>0.08</td>
<td>77.3</td>
<td>19 (± 1)</td>
<td>39 (± 26)</td>
</tr>
<tr>
<td>0.16</td>
<td>154.6</td>
<td>22 (± 7)</td>
<td>24 (± 1)</td>
</tr>
<tr>
<td>0.32</td>
<td>309.2</td>
<td>14 (± 3)</td>
<td>34 (± 1)</td>
</tr>
</tbody>
</table>
Figure 1: Effect of lignocaine in combination with DAC, and effects of bupivacaine and ropivacaine on MCF-7 and BT-20 human breast cancer cells. The cell viability was analyzed by MTT analysis after being cultured for 72 h with the indicated drugs. (A) BT-20 cells and (B) MCF-7 cells treated with lignocaine and various concentrations of DAC. (C) BT-20 cells and (D) MCF-7 cells treated with bupivacaine and ropivacaine. Data are represented as the mean ± SD from three independent experiments.

Figure 2: Global Genomic SAT2 DNA methylation analysis in breast cancer cell lines after treatment with bupivacaine and ropivacaine. (A) SAT2 DNA methylation levels in BT-20 breast cancer cells treated with 2 µM and 20 µM bupivacaine, 3 µM and 30 µM ropivacaine or 1 µM DAC and (B) MCF-7 breast cancer cells treated with 2 µM bupivacaine, 3 µM and 30 µM ropivacaine or 1 µM DAC. Results from an average of 6 independent experiments are shown. Results are expressed as mean ± SD. Statistical significance between control and treated samples was assessed by Mann-Whitney U test (*, p < 0.05; **, p < 0.01;***, p < 0.001).

Figure 3: Global Genomic SAT2 DNA methylation analysis in breast cancer cell lines after combined treatment of lignocaine, bupivacaine and ropivacaine with various concentrations of DAC. (A) SAT2 DNA methylation levels in BT-20 breast cancer cells and (B) MCF-7 breast cancer cells. Results from an average of 6 independent experiments are shown. Results are expressed as mean ± SD. Statistical significance between control and treated samples was assessed by Mann-Whitney U test (*, p < 0.05; **, p < 0.01).

Figure 4: Line of additivity based on the preliminary calculated ED50 for DAC and Lignocaine (0.08 µM and 77.3 µM, respectively). The marker denotes the first concentration which leads to a near-50% demethylation (actual ED50).
Demethylating properties of lidocaine, bupivacaine and ropivacaine

Figure 1
Figure 2

A

B

Chapter 4.2

266
Figure 3

(A) BT-20

(B) MCF-7

Sae2 DNA methylation (RAI values)
Figure 4

![Graph showing DAC (µM) vs. Lignocaine (µm) with a line of additivity indicated.](image)
Conclusion and Discussion: Weighing risks and benefits of Regional Anaesthesia

Based on

Lirk P, Hollmann MW

Regional anesthesia: weighing risks and benefits

Minerva Anesthesiol 2013; epub ahead of print Nov 6
Introduction

During the past 30 years, regional anaesthesia has undergone dramatic changes in technique and indications, and has become a widely used method to provide intraoperative anaesthesia, and postoperative analgesia. A considerable base of scientific evidence has been achieved from which to estimate risks and benefits of regional anaesthesia, including the insight that the failure rate of regional anaesthesia in daily practice may be high. Evidence-based medicine seeks to combine these scientific results with pathophysiological knowledge and the needs of the individual patient to devise a judicious anaesthetic plan. This review seeks to address the question whether patient outcomes are sufficiently improved by regional anaesthesia to an extent that warrants its use as a routine perioperative method.

The benefits

In the past, the perceived ability of regional anaesthesia to provide excellent management of acute postoperative pain combined with a good safety profile was considered a sufficient reason in itself to perform regional anaesthesia. Indeed, many authors continue to appreciate the value of good perioperative pain therapy as such, even if it cannot be directly translated into improved overall long-term outcome. However, recent literature has advocated a more rigorous judgement of the value of regional anaesthesia. With the advent of improved general anaesthesia regimens and multimodal pain therapy, the superiority of regional anaesthesia in many types of surgery has receded to levels that may be statistically, but not clinically, significant. The benchmarks against which the efficacy of regional anaesthesia should be measured were summarized by Fischer (2010): optimal postoperative analgesia with a minimum of adverse effects, promotion of rehabilitative efforts, and improvement of long-term outcome including chronic pain. The problematic nature of these bars was highlighted in a recent editorial. Specifically, regional anaesthesia is but one, albeit important, therapeutic option during the perioperative course of an individual patient. In the optimal evidence-based situation, all patient- and procedure-related factors and interventions should be efficient in altering overall outcome. In an age where increasing numbers of patients are in fast-track discharge programs and
Weighing risks and benefits

perioperative risk of major morbidity and mortality is low, respective
benefits of regional anaesthesia become harder to discern.

Acute and Chronic Pain

Poorly managed acute postoperative pain is, in general, thought to be
associated with the development of chronic pain. However, it is difficult to predict
why certain patients go on to develop chronic pain while in others, pain resolves.
Part of this risk is determined genetically. The respective concept is that of an
“estimated heritability score”. In the case of acute pain, the risk “share” of genetic
determinism is between 22-55%. In contrast, the heritable component for the risk
of developing persistent pain is higher, in the range of 55-68%. Such data clearly
suggest a genetic predisposition for those who develop chronic pain following a
precipitating incident. Pain which is difficult to control perioperatively may reflect
greater sensitivity of patients based on genetic predisposition, with chronic pain as a
logical consequence. Strict regimens of pain therapy may help to reduce chronic
pain after prototypical high-risk, such as amputation. In the latter scenario, use of
epidural anesthesia or peripheral nerve blocks may substantially reduce the rate of
phantom limb pain, but the evidence-base is limited at this point. The prolonged use
of peripheral sciatic nerve block for amputation was shown in recent reports to have
a high success rate in preventing phantom limb pain. Potentially, future studies
will make it possible to identify individuals at high risk of acute and chronic pain,
and to tailor individual patient-specific treatment plans including the provision of
regional anaesthesia, according to both type of surgery and type of patient. In
patients at high risk of excessive pain perception undergoing major surgery, these
treatment plans may include the combination of a sufficient regional blockade
together with intravenous multimodal pain treatment (intravenous non-opiates,
combined with, e.g., systemic ketamine, clonidine, or gabapentin / pregabaline).
This would act to prevent the development of central sensitization via different
pathways.
Peripheral Nerve Blocks

An exhaustive analysis by Liu and Wu examined the beneficial effects of regional anaesthesia when compared to systemic analgesia. In general, the authors found an average acute pain score with regional anaesthesia which was only marginally lower, and a lack of clear evidence showing improved long-term outcome. Despite the fact that many studies report statistically significant outcomes, a clear benefit in pain scores should only be assumed and taken as clinically relevant when the absolute pain intensity is different by 2 points on a 10-point scale, or pain differs by 33\%. For specific surgeries, such as joint replacement, regional anaesthetic techniques such as single-shot or continuous peripheral nerve blocks are widely used, with an excellent safety profile and a substantial reduction in opioid administration. This was confirmed in recent meta-analyses and systematic reviews demonstrating superior analgesia, less opioid-induced side-effects and sleep disturbances, and facilitated rehabilitation when continuous nerve blocks were used for extremity surgery. The timepoint and definition of analgesic endpoint is important, as well. For example, the meta-analysis by Richman investigating continuous nerve blocks as compared to opioids showed that the mean VAS score was reduced from 3.7 to 1.7, on the verge of clinical significance. However, when maximal pain scores at breakthrough points were considered, the reduction was more pronounced, from 5.9 to 3.2.

In the future, alternative blockades such as truncal blocks may replace epidural anaesthesia for some indications, but the evidence is insufficient at this moment. Early promising studies show that transversus abdominis plane block may promote short-term recovery after abdominal surgery, and may be an alternative to spinal morphine after caesarean section. Current literature regarding paravertebral blockade shows a trend towards equally effective analgesia as compared to the epidural route with potentially less adverse effects.

Epidural anaesthesia

Considering epidural anaesthesia for upper abdominal and thoracic surgery, Andreae found a beneficial effect of regional anaesthesia on development of chronic
Weighing risks and benefits

pain after open thoracotomy and breast cancer surgery but stated that in general, studies were of suboptimal quality and had included few patients. The combination of intraoperative epidural and multimodal analgesia has been found to prevent chronic pain after major abdominal surgery more effectively than multimodal analgesia alone. Moreover, patients undergoing major vascular surgery, and high-risk patients, have been found to experience less perioperative complications when general is combined with epidural anaesthesia, but the reported effect size is small. For example, epidural administration of local anaesthetics protects against postoperative ileus, but beneficial effects upon gastrointestinal motility have also been published regarding the intravenous administration of local anaesthetics. The same ambiguity also holds true for other outcomes. Whereas Liu did not observe a difference in quality of recovery, another meta-analysis by Poepping, including older studies, postulates that after upper abdominal and thoracic surgery, epidural anaesthesia decreases the risk of pulmonary complications. Notably, in the latter study, the incidence of pulmonary complications in patients receiving epidural anesthesia remained steady at 8% throughout the meta-analysis inclusion range, whereas the incidence in the systemic analgesia group decreased from 34% to 12%, most likely reflecting improved supportive perioperative care, and higher efficacy of multimodal systemic regimens. On a broader basis, it can be stated that the quality of multimodal intravenous regimens and perioperative care is improving, and it can be speculated that studies comparing the effects of epidural anaesthesia against optimal intravenous regimens will demonstrate beneficial effects on a much smaller scale. A recent Cochrane review failed to show a decrease in mortality in patients undergoing open abdominal aneurysm surgery, but the use of epidural analgesia reduced pain during the three first postoperative days (an arguable clinical benefit if only seen by itself), decreased postoperative ventilation time by half, and led to a reduction in postoperative myocardial infarction, acute respiratory failure, and gastrointestinal and renal complications. It can be summarized that epidural anesthesia and analgesia improve aspects of postoperative outcomes in selected patient subgroups. Contemporary medicine is facing two distinct but opposing trends. On one hand, evidence-based medicine relies on large-scale investigations
investigating aspects of patient care, and when large patient collectives are considered, the averaged effect size of epidural anaesthesia across all patients is small to modest. On the other hand, the future trend is that of personalized, individually tailored, medicine, based on, among others, the patient’s previous medical and pain history, and genetic background. This trend is certain to encompass the areas of perioperative and chronic pain medicine, and will lead to a further refinement of the indications for neuraxial blockade.

**Regional Anaesthesia and Cancer Medicine**

Whether regional anaesthesia has the potential to improve outcome in cancer patients has been the topic of intense discussion and research efforts ever since a retrospective study demonstrated survival benefits in patients subjected to perioperative paravertebral anaesthesia during mastectomy. Since then, some studies have confirmed this promising trend, others have failed to detect beneficial effects of regional anaesthesia, and some have found benefits only in specific patient subgroups. The mechanisms by which regional anesthesia may theoretically affect tumour progression are threefold. First, perioperative suppression of the surgical stress response by regional anaesthesia improves host defence, and may protect against circulating tumour cells. Second, opioids have arguably been linked to promote tumour growth in some experimental models and regional anaesthesia allows for dose-minimization of opiates, but it is unclear whether opiates clinically affect cancer recurrence. Third, local anesthetics present in the systemic circulation may directly limit viability of tumour cells, or sensitize them against chemotherapeutics. If the large-scale studies currently ongoing do find a clinically relevant effect of regional anaesthesia, it can be expected to be valid for certain types of tumour only, and depend on the molecular biological background of the specific malignancy.

**Systemic Effects of Local Anaesthetics**

Lastly, it should be mentioned that local anaesthetics have effects that reach beyond “just” nerve blockade and ensuing pain control. Among others, they have
been shown to beneficially affect the postoperative surgical stress response without impairing physiologic host response. Further, they have been shown to prevent hypercoagulable states in patients after major vascular and orthopaedic surgery without increasing the risk of bleeding. When compared to placebo, systemic administration of lidocaine has been reported to reduce opioid consumption and related side effects, decrease duration of ileus, and decrease length of hospital stay following abdominal surgery. Notably, these beneficial effects are observed after both open and laparoscopic surgery.

When comparing systemic lidocaine to epidural anesthesia as the conventional “gold standard” for major abdominal surgery, the results are less straightforward. Intravenous lidocaine started at the beginning of surgery has similar effects as epidural analgesia started within one hour after surgery regarding duration of ileus, pain, and length of hospital stay. However, in the latter study, initiation of the epidural was performed only after surgery, and was not standardized, even though other studies suggest that the main positive effect of epidural anesthesia and analgesia is attained intraoperatively. Another study compared intraoperative administration of thoracic epidural anesthesia, systemic lidocaine, or placebo. Postoperatively, all patients were connected to patient-controlled epidural analgesia (PCEA). Benefits on pain were short-lived after surgery as patients were allowed to administer boluses of epidural solution themselves. However, beneficial effects on duration of ileus, and the decrease in inflammatory mediators were more evident in patients who had received epidural than in patients who had received intravenous local anesthetics, and in both groups, functional outcome, but not length of hospital stay, was improved as compared to intraoperative placebo.

Adverse effects of intravenous lidocaine primarily stem from systemic toxicity, and most studies report plasma levels well below the toxic threshold of 5 µM. Signs of central nervous system toxicity are seldomly reported.

The systemic action of local anaesthetics is dependent on sufficient plasma levels usually reached during neuraxial block, peripheral nerve blockade, and intravenous administration. However, recent advances in the administration of ultrasound-guided regional anaesthesia have led to descriptions of block success in
the presence of dramatic reductions in the volume of administered local anaesthetic. Some reported volumes are less than 10% of the “traditional” volume administered when using nerve stimulation.\textsuperscript{35} Using very small volumes for peripheral nerve block is theoretically advantageous because of the decreased danger of systemic local anaesthetic toxicity, but at the same time, the systemic beneficial effects of regional anaesthesia, whose contribution is only beginning to be fully understood, will be lost because systemic absorption and resulting plasma levels are negligible.

In conclusion, beneficial effects of regional anaesthesia can be copied to a large extent by the systemic administration of local anesthetics. Considering the risk:benefit ratio, epidural anesthesia and analgesia should be considered in high risk patients, thoracotomy, and major upper abdominal and vascular surgery, whereas most cases of lower abdominal surgery and laparoscopic surgery can be adequately treated using multimodal analgesia.

The risks

When considering the risk aspect of regional anaesthesia, neurologic complications such as peripheral nerve injury or paralysis, and systemic complications such as local anaesthetic intoxication are the most commonly cited major adverse outcomes.

Permanent nerve injury

The incidence of perioperative peripheral nerve injuries has been assessed in a variety of studies and in general, retrospective studies show a much lower incidence than prospective studies. A summary of clinical studies by Brull et al. found a rate of neurologic complications of 0.04 % after neuraxial, and 3 % after peripheral regional anaesthesia, with the majority of neurologic deficits transient in nature.\textsuperscript{36} In a recent analysis of 1000 ultrasound-guided peripheral nerve blocks, the incidence of all-cause new-onset neurologic symptoms was 8.2%, with most cases unrelated to the block after thorough analysis.\textsuperscript{37} Since then, prospective data in 8,000 epidurals showed a complication rate for abscess formation of 0.1%.\textsuperscript{38} The Third National Audit Project of the Royal College of Anaesthetists reported
permanent disability or death from central nervous blockade between 0.7 and 1.8 per 100,000 procedures.\textsuperscript{39} The question whether regional anaesthesia is more dangerous in patients with pre-existing neurological disease has been controversially discussed.\textsuperscript{40} Current evidence suggests that symptomatic diabetic neuropathy may warrant a more careful weighing of the indication for regional anaesthesia, and may necessitate adaptation of technique, such as a decrease in local anaesthetic dose, and decreasing or omitting epinephrine from the injectate.\textsuperscript{40}

\textit{Systemic Toxicity}

Regional anaesthesia can further be complicated by systemic overdose of local anaesthetic. This potentially life-threatening adverse event occurs after inadvertent intra-arterial injection, intra-venous injection, or absorption following large-volume blocks.\textsuperscript{41} In the landmark study by Auroy and colleagues, seizures as indicators of systemic toxicity occurred in 1 / 10,000 patients undergoing epidural or spinal anaesthesia, and between 2 and 5 / 10,000 patients undergoing peripheral nerve blocks.\textsuperscript{42} Recently, ultrasound-guided peripheral regional anaesthesia was reported to cause seizures in 8 / 10,000 patients, with no incidences of cardiac arrest in a collective of more than 12,000 patients.\textsuperscript{43} Due to increased awareness and multiple safety steps such as aspiration, careful titration of the local anaesthetic, and the administration of test doses, the incidence of systemic toxicity is estimated to have declined by a factor of 25.\textsuperscript{41} In addition, intralipid has been introduced as a possible therapeutic adjunct for intoxication due to lipophilic local anaesthetics such as bupivacaine.\textsuperscript{44}

It should be briefly noted, however, that opiates as the main alternative substance for postoperative pain relief, can cause severe adverse effects as well, including respiratory depression and arrest. Together with anticoagulants and insulin, opiates are among the three most common substances to cause serious adverse drug events in hospitals.\textsuperscript{45}
Other adverse events

Several other potential adverse events need to be considered on a patient- and block-specific basis. Pneumothorax has been reported after several upper extremity nerve blocks, including interscalene, supraclavicular, and vertical infraclavicular. Vascular puncture can occur, with hematoma formation or, very rarely, pseudoaneurysm formation as sequelae. While this is perceived to be less of a problem in peripheral regional anesthesia, epidural hematoma formation may lead to compression of neural structures, and to permanent damage, with an incidence of 1:4000 – 1:6000 in the general surgical population, and less in obstetrical patients. Similarly, infection localized to the site of peripheral nerve block may be troublesome, but development of an epidural abscess may precipitate permanent neurologic dysfunction if occurring in the spinal canal. Moreover, excessive spread of local anaesthetic can lead to inadvertent blockade of nerves other than the target structure. Vocal cord paralysis can ensue due to recurrent laryngeal nerve block, and hemidiaphragmatic paresis can occur due to phrenic nerve block after interscalene or supraclavicular nerve block. These side-effects may potentially be avoided by using small volumes during ultrasound-guided nerve blockade. Again, these latter side-effects may be of limited clinical importance in healthy patients, but they may be detrimental in patients with specific co-morbidities, e.g., contralateral recurrent nerve palsy or severe chronic obstructive pulmonary disease, respectively.

Weighing the risks and benefits

At the moment, regional anaesthesia is undergoing a profound transition. The current trend is to reconsider and reappraise indications and contraindications for regional anaesthesia, often in the light of changing surgical techniques. One paradigmatic example is analgesia for total knee arthroplasty (TKA). Regional anaesthesia has been demonstrated to offer substantial benefits in the immediate perioperative period of TKA as compared to systemic analgesia, including superior pain control, reduced opioid consumption, and decreased opioid-related side-effects. In the middle term, ambulation and range of motion is improved in patients receiving regional anaesthesia groups, and rehabilitation goals are met faster. To
achieve this goal, some twenty years ago, it was considered good clinical practice to perform epidural analgesia or combined spinal-epidural anaesthesia and analgesia. However, epidural analgesia is frequently associated with pruritus, urinary retention and motor block, hindering optimal and early mobilization. Subsequent evidence demonstrated that a combined femoral / sciatic nerve block would provide the same degree of postoperative analgesia, but with a more favourable side-effect profile, especially concerning early mobilization. Currently, the two-block approach is undergoing scrutiny, and in many patients, continuous femoral nerve block (possibly combined with a single-shot sciatic nerve block) seems to provide satisfactory analgesia. Newer techniques such as saphenous nerve block seem even more tailor-made for analgesia following TKA. This nerve block provides analgesia for the anterior portion of the knee joint, while avoiding blockade of motor fibers such as those supplying the quadriceps muscle. In theory, mid-thigh saphenous block will combine the analgesia of a femoral nerve block but without the motor weakness potentially interfering with early rehabilitative efforts, providing targeted analgesia as peripherally as possible.

However, in the long term, outcome after one year following TKA is not as clear-cut. Whereas immediate postoperative benefits, including improved pain control and less adverse opioid-induced adverse effects can readily be detected in trials, functional patient outcome one year after surgery is not superior. With the current cost-driven trend to fast-track TKA patients, it becomes increasingly difficult to demonstrate outcomes such as reduced length of stay. It is debatable whether the facilitation of rehabilitation, which has been clearly demonstrated is a sufficient reason to perform regional anaesthesia if one-year functional outcome is not superior. The authors think that the combination of good perioperative pain therapy, decrease of opioid-related adverse events, and facilitated rehabilitation should be considered sufficient grounds to continue offering peripheral regional anaesthesia to patients undergoing TKA.
Future directions

Evolving research fields may alter the way we look at perioperative interventions, including regional anaesthesia. For example, epigenetics is concerned with the methylation of desoxyribonucleic acid (DNA), which determines gene expression. An increasing number of pathological conditions, among them persistence of experimental pain,\(^57\) has recently been linked to epigenetic irregularities. Interestingly, chronic opioid use in patients leads to increased DNA methylation correlating with increased pain, at least in part explaining opioid-induced hyperalgesia.\(^58\) The effect of clinically relevant concentrations of lidocaine has the opposing effect on methylation in vitro, i.e. de-methylation.\(^26\) Whether this molecular effect can be translated into strategies to decrease acute pain and prevent chronic pain needs to be determined at this point.

In parallel, the tools used to deliver Regional Anesthesia are enhanced. One very prominent example is the new liposomal formulation of bupivacaine, which is entering the United States market, and may extend pain relief to 48-72 hours after a single bolus injection.\(^59\) The efficacy and safety of this treatment modality will undoubtedly be the subject of a large number of studies.

Conclusion

In conclusion, the question whether regional anaesthesia generally improves relevant indices of outcome in all patients has to be answered with no. Research has led to the reconsideration of indications and contraindications for regional anaesthesia in specific settings. The administration of regional anaesthesia necessitates a judicious weighing of risks and benefits. In general, the indications for epidural anaesthesia have decreased, and are limited to major upper abdominal and major vascular surgery, and thoracotomy. In addition, patients might benefit from regional anaesthesia on an individualized specific risk:benefit deliberation. It should be noted that when broad patient collectives are considered, the benefits of epidural anaesthesia are modest. Peripheral nerve blockade remains a mainstay of anaesthetic management for extremity surgery. At this time, there is insufficient evidence to ascribe a specific role to alternative blockades such as truncal blocks, but in the
future, they may replace epidural anaesthesia for some indications. Paravertebral anaesthesia may evolve into an alternative to epidural anaesthesia for thoracotomy.

A substantial share of the alternative effects of regional anaesthesia on the immune system, postoperative ileus, and haemostasis are due to systemic absorption of local anaesthetics, and can be duplicated using intravenous administration of local anaesthetics. Low-volume peripheral nerve blocks may lack these beneficial effects because plasma levels are negligible. Multimodal analgesia has the potential to replace regional anaesthesia in many types of surgery. Regional anaesthesia continues to play a major role in perioperative medicine, but its role keeps getting more defined and less noncommittal.

Key messages

- Every regional blockade should be preceded by a weighing of potential risks and proven benefits, taking into account procedure- and patient-based factors.
- At least in part, many beneficial effects of Regional Anaesthesia can be duplicated by intravenous administration of local anaesthetics.
- Regional anaesthesia continues to play a major role in perioperative medicine, but its role keeps getting more defined and less noncommittal.

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Summary

Section 6
The present thesis sought to investigate and review the mechanism and management of side-effects or failures of neuraxial anesthesia; investigate nerve injury related to the performance of regional anesthesia, with special focus on patients with pre-existing neuropathy; investigate potential novel actions of local anesthetics on the epigenetic signature of tumour cells; and conclude with a weighing of risks and benefits of regional anesthesia using local anesthetics.

In Section 1, an introduction was given to local anesthetics. While Chapter 1.1 described the aims of this thesis, Chapter 1.2 reviewed current literature which shows that conventional local anesthetics in contemporary use block the voltage-gated sodium channel by binding to a specific site on the inner facet of the channel pore. This causes a conformational change, and the positive charge of the local anaesthetic further obstructs the channel’s lumen. Only 2-3% of local anaesthetic is thought to participate in nerve blockade, the rest is absorbed into surrounding tissues or the systemic circulation. While small systemic levels of local anaesthetic can have a beneficial effect on some outcomes and vital functions, excessive amounts of free local anaesthetic will cause systemic toxicity.

In Section 2, focusing on the clinical application of local anaesthetics for regional anesthesia, two studies concentrated on the use for spinal anesthesia for cesarean section, while one review article described causes and management of failed neuraxial blockade. In Chapter 2.1, we present a survey investigating the strategies to prevent or treat hypotension following administration of spinal anaesthesia for caesarean delivery. In comparison to previous surveys in the United States and United Kingdom, we showed a trend toward increased use of phenylephrine to treat hypotension, in keeping with a growing evidence-base suggesting its side-effect profile is superior to ephedrine in most cases. In Chapter 2.2, we investigated pulmonary function indices following administration of spinal anesthesia for caesarean section when using bupivacaine, ropivacaine or levobupivacaine. We found that decreases in maternal pulmonary function tests were similar following spinal anaesthesia with bupivacaine, ropivacaine, or levobupivacaine for caesarean delivery. The clinical maternal and neonatal effects of these alterations appeared negligible. This study does not support the clinical
superiority of any of the three investigated long-acting local anaesthetic for spinal anesthesia. In *Chapter 2.3*, the evidence surrounding failures and their management during neuraxial analgesia is reviewed. Failed epidural anaesthesia or analgesia is more frequent than generally recognized. Reasons for an insufficient epidural include primary erroneous placement, secondary migration of a catheter after correct placement, and suboptimal dosing of drugs. As hinted in *Chapter 2.2.*, when using equipotent doses, the clinical difference between bupivacaine and the newer isoforms levobupivacaine and ropivacaine appears minimal. During continuous infusion, dose is the primary determinant of epidural anaesthesia quality, with volume and concentration playing a subordinate role. Addition of adjuvants, especially opiates and epinephrine, may substantially increase the success rate of epidural analgesia. The method of delivery for postoperative analgesia most supported by literature is patient controlled epidural analgesia with background infusion.

In *Section 3*, the focus was on nerve damage during regional anesthesia, with a special focus on patients with diabetic neuropathy. In *Chapter 3.1*, we sought to answer the question whether intraneural needle placement is detrimental. In a large animal model of sciatic nerve block, neurophysiological parameters following nerve trauma and intraneural injection. Needle trauma as well as intraneural injection of small volumes of saline produced a significant decline in cMAP amplitude, corresponding to severely impaired nerve function. Although the degree of long-term neurological dysfunction in our model is unclear, it could be demonstrated that in the short term, nerve conduction is highly vulnerable to needle trauma and intraneural injection, arguing against intraneural injection as proposed by some authors. In *Chapter 3.2*, current knowledge on regional anesthesia in patients with diabetic neuropathy was summarized. Patients with pre-existing neuropathies or neurological disease may be at increased risk of iatrogenic nerve injury. This line of thought was developed further in *Chapter 3.3.*, where in vitro and in vivo investigations were conducted to investigate effects of local anesthetics on nerve block duration and neurotoxicity in diabetic rats. We found that *in vitro*, local anesthetic neurotoxicity was more pronounced on neurons from diabetic animals,
but the survival difference was small. *In vivo*, subclinical neuropathy leads to substantial prolongation of block duration. We concluded that early diabetic neuropathy increases block duration, while the observed increase in toxicity was small. In a subsequent experiment (*Chapter 3.4*), we refined this methodology by introducing neurophysiologic monitoring to diagnose diabetic neuropathy before administering sciatic nerve block and measuring nerve block duration as well as neurotoxic effects. We found that nerve blocks last longer in late diabetic animals. Sensitivity of diabetic nerves for local anesthetics seems to correlate with severity of neuropathy. Our results, using a short-acting local anesthetic without additives, do not support the hypothesis that nerve block in diabetic patients increases nerve injury after peripheral nerve block. Lastly, in *Chapter 3.5*, we sought to assess the patterns of practice in the management of diabetic neuropathic patients presenting for regional anesthesia in Europe. While literature is divided on the question whether pre-existing diabetic neuropathy is a risk factor for new neurological deficit after regional anaesthesia, most of the responders of this survey take measures to reduce risks, counsel patients on a possible greater risk of neurologic complications, but only a minority of responders would avoid peripheral regional anaesthesia altogether.

**Section 4** concentrates on a novel mechanism of action of local anesthetics, the modification of the epigenetic signature of human cells. While preliminary evidence had suggested that procaine can modify epigenetics in tumour cells, we could show in *Chapter 4.1* that this was also the case for the classic local anesthetic, lidocaine, at clinically relevant concentrations attained after epidural anesthesia or intravenous administration as part of multimodal pain treatment regimens. In *Chapter 4.2*, we show that lidocaine and ropivacaine, but not bupivacaine, at clinically relevant doses, exert demethylating effects on certain breast cancer cells, and seem to enhance demethylating properties of prototype epigenetic chemotherapeutic, 5-aza, in an additive fashion. At equipotent doses, the demethylating effects of local anesthetics seem to be most pronounced for lidocaine, with decreased demethylating capacity by ropivacaine, and no detectable effect of
bupivacaine. These effects could be of substantial importance in perioperative medicine, with focus on tumour surgery and pain medicine.

In Section 5, the potential benefits and risks of regional anesthesia were compared. Regional anesthesia has become a widely used method to provide intraoperative anesthesia, and postoperative analgesia. This review seeks to address the question whether patient outcomes are improved to an extent that justifies using regional anesthesia as a routine method. During the past decade, a very critical appraisal of risks and benefits of regional anaesthetic procedures has taken place. In general, the indications for epidural blockade have decreased, and are limited to individual high-risk patients, major upper abdominal and major vascular surgery, and thoracotomy. We review the changing role of central and peripheral regional anesthesia in the perioperative management of total knee arthroplasty. Immediate perioperative outcome after knee arthroplasty concerning function and pain is improved, and rehabilitation facilitated, by peripheral nerve blockade, but this does not translate into superior functional outcome one year later. A substantial share of the beneficial effects of regional anesthesia on the immune system, haemostasis, pain, and the duration of ileus can be duplicated using intravenous administration of local anaesthetics. In general, the use of regional anesthesia should always be preceded by a weighing of potential risks and proven benefits. Regional anesthesia continues to play a major role in perioperative medicine, but its role keeps getting more defined and less noncommittal.
Appendices
Dutch summary / Nederlandse samenvatting
Dit proefschrift getiteld “Local anesthetics – new insights into risks and benefits” heeft als primaire doelstelling om specifieke bijwerkingen van de regionale anesthesie te onderzoeken. Daarnaast wordt de invloed van diabetische neuropathie op toxiciteit en functioneren van een perifeer zenuwblok omschreven, en het potentiële effect van lokaal-anesthetica op de epigenetische markering van tumorcellen bepaald. In het laatste hoofdstuk zullen de risico’s en baten van de regionale anesthesie worden beschreven.


De huidige discussie in de anesthesie heeft betrekking op de invloed van een preexistente neuropathie op het functioneren van lokaal-anesthetica. Ons onderzoek suggereert dat het risico op zenuwschade door regionale anesthesie bij reeds bestaande diabetische neuropathie niet significant toeneemt. Maar ons onderzoek laat ook zien dat de zenuwblokade in dit geval veel langer duurt dan bij gezonde zenuwen. De zenuwen van diabetische patiënten zijn dus toch gevoeliger voor lokaal-anesthetica ook al lijkt het risico op permanente beschadiging door regionale anesthesie niet verhoogd. In de klinische praktijk is er veel onduidelijkheid hoe er met diabetische patiënten met preexistente zenuwschade om moet worden gegaan. Een van de conclusies in dit proefschrift is dat een regionaal-blok verantwoord is mits een goede afweging van risico’s en baten plaats heeft gevonden.

Lokaal-anesthetica hebben naast de blokkade van het natrium-kanaal nog andere, alternatieve, effecten. Wij zijn de eersten die aantonen dat lokale verdovingsmiddelen in klinisch relevante concentraties ook in staat zijn om in vitro de epigenetische markering in cellen te veranderen. Verder onderzoek naar dit fenomeen is noodzakelijk.
Als laatste bespreken wij de risico’s en baten van regionale anesthesie in de gehele populatie. Regionale anesthesie ondergaat momenteel een belangrijke verandering. Steeds meer indicaties voor specifieke technieken worden herzien of komen te vervallen. Andere indicaties worden juist sterker. Al in al is het gebruik van regionale anesthesie niet meer zo vrijblijvend als tien jaar geleden. Een duidelijke afweging van risico’s en baten moet aan elke regionale techniek voorafgaan.
Curriculum vitae of the candidate
Curriculum vitae

Date of birth February 22nd, 1977 in Salzburg, Austria.

Nationality Austrian.

Marital status Married to Petra Lirk-Heinrich, 2 sons Ferdinand and Leopold.

Languages spoken German, Dutch, English, French.

- 1995: Reifepruefung, Matura, Hoehere Internatsschule des Bundes, Saalfelden, Austria
- 2001-2006: M.Sc. in Anesthesiology (Pain Management), University of Wales College of Medicine, United Kingdom. Title of dissertation: Role of calcium channels in a rodent model of chronic neuropathic pain.
- July 2003 – August 2004 Postdoctoral Research Fellowship at the Dept. of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA.
- November 2002 – April 2010 Residency in Anesthesiology and Critical Care Medicine, Innsbruck Medical University, Austria.
- April 2010 Austrian Board certification in Anesthesiology and Critical Care Medicine.
- May 2010 Venia docendi (Postgraduate degree, Privatdozent), Innsbruck Medical University, Austria.
- August 2010 – present Universitair Medisch Specialist (Attending Anaesthesiologist) at the Academic Medical Center, University of Amsterdam.
- July 2011 – July 2016 Associate Professor at the University of North Florida Brooks College of Health, Jacksonville, FL, USA.
Grants, Awards and Prizes

- Medical Research Foundation Grant 103a (Innsbruck Medical University) 2004
- Midwest Anesthesia Resident Conference Second Best Poster Prize 2004
- Swarovski Research Award (Innsbruck Medical University) 2005
- ESRA Annual Meeting Best Free Paper Prize 2005 (First author)
- ESRA Annual Meeting 2nd Best Free Paper Prize 2006 (Senior author)
- ESRA Annual Meeting Best Poster Prize 2006 (Presenting author)
- ESRA Annual Meeting 3rd Best Free Paper Prize 2007 (Co-author)
- ESRA Annual Meeting Best Poster Prize 2011 (First author)
- Academic Medical Center Patient Care Quality Improvement Grant 2011
- Dutch Society of Anaesthesiology Young Investigator’s Award 2012
- European Society of Anaesthesiology Clinical Trial Network Award 2012
- Dutch Society of Anaesthesiology Ritsema van Eck Award 2nd Prize 2013
- European Society of Regional Anaesthesia Research Grant 2013

Publications - Book chapter

Publications - Peer-reviewed
- Number of publications \( n = 74 \), 2 in press, 2 in revision.
- Number of times cited: 1070 (ISI Web of Knowledge, accession date 02.05.2014)
List of publications of the candidate
Publication list

Publications in revision

Publications in press

Peer-reviewed publications
1 Picardi S, Stevens MF, Hahnenkamp K, Durieux ME, Lirk P, Hollmann MW. Time-dependent modulation of muscarinic m1/m3 receptor signalling by local anaesthetics. *Br J Anaesth* 2014; 112: 370-9

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Research portfolio of the candidate
**Grants, Prizes and Awards during Graduate Studies**

- ESRA Annual Meeting Best Poster Prize 2011 (First author)
- Academic Medical Center Patient Care Quality Improvement Grant 2011
- Dutch Society of Anaesthesiology Young Investigator’s Award 2012
- European Society of Anaesthesiology Clinical Trial Network Award 2012
- NVA Ritsema van Eck Award 2nd Prize 2013 (First author)
- European Society of Regional Anaesthesia Research Grant 2013

**Courses during Graduate Studies**

- Good Clinical Practice (GCP) course 2011
- Animal experimental course FELASA-C (Art 9 cursus) 2011
- Basiskurs Regelgeving en Organisatie voor Klinische Onderzoekers (BROK) 2013
- Reviewer workshop, British Journal of Anaesthesiology, 2013

**Invited lectures (selection) during Graduate Studies**

- *Management of the elective cesarean section.* Austrian Congress of Anesthesiology, Resuscitation and Intensive Care Medicine, Klagenfurt, Austria, September 2012
- *Refresher course: local anesthetics.* European Society of Regional Anesthesia and Pain Therapy (ESRA) Annual Congress, Bordeaux, France, September 2012
- *Intraneural injection: no!* European Society of Regional Anesthesia and Pain Therapy (ESRA) Winter Week, Grindelwald, Switzerland, January 2013
- *Local anesthetic neuro- and myotoxicity.* European Society of Regional Anesthesia and Pain Therapy (ESRA) Winter Week, Grindelwald, Switzerland, January 2013

Ultrasound and what it has taught us about Regional anesthesia. University of North Florida Patient Safety Conference, Jacksonville, FL, USA, March 2013

Peripheral neuropathy and Peripheral nerve block. Americal Society of Anesthesiology Annual Congress, San Francisco, CA, October 2013

Local anesthetics: from basic research to new clinical applications. Vinzenztage Congress, Vienna, Austria, November 2013


Local anesthetics demethylate DNA in breast cancer cells in vitro. Clinical Epigenetics Society, Dusseldorf, Germany, March 2014


Peripheral Nerve block in Diabetic Neuropathy. Morning Rounds, Hospital for Joint Diseases, New York University Medical Center, New York, NY, USA, March 2014

Epigenetics and local anesthetics. Symposium Neural Therapy, University of Heidelberg, Germany, May 2014

Big data. Dutch Society of Anesthesiology Annual Congress, Maastricht, May 2014

Local anaesthetics: the essentials. European Society of Anaesthesiology Annual Congress, Stockholm, Sweden, June 2014

Editorial services

Regular reviewer for the journals Anesthesia & Analgesia, Minerva anestesiologica, and the British Journal of Anaesthesia.

Service on committees

Member, European Society of Anaesthesiology (ESA) Scientific Subcommittee 8 (Regional Anesthesia) 2014-2016
Acknowledgment / Dankwoord
The last “opdracht” of this Thesis is a truly pleasurable one: to express gratitude to the many people who each contributed such important pieces to the Work as it stands now.

The first thanks traditionally go out to the Promotor, and that is especially fitting for this Thesis. I would like to thank Prof. Markus Hollmann for welcoming me into the Department of Anesthesiology at the Academic Medical Center (AMC), and for giving me so much inspiration, support and liberty on my Academic path. My second gratitude is expressed to my Department Chair Prof. Wolfgang Schlack, for giving me the opportunity to come to the AMC, and for having the vision that Amsterdam would be a good place for my family and me. Thank you so much. It is.

To the two co-promotores on this Thesis, Nina Hauck-Weber and Markus Stevens, I would like to express my gratitude for their enormous and invaluable support in all things laboratory, experimental, academic and Dutch. Their advice and knowledge of Holland, Amsterdam and the AMC contributed greatly to making the start back in 2010 a very positive and rewarding one.

I would like to thank all the members of the Graduation Committee, Prof. Wolfgang Schlack, Prof. Ivo van Schaik, Prof. Marcus Schultz, Prof. Peter Gerner, Prof. Jim Rathmell, dr. Geert-Jan van Geffen, and Dr. Lukas Kirchmair, for their willingness to grade the Thesis, and to come to the Agnietenkapel on June 11th 2014 to enter into an academic discussion.

This work has been the product of a fruitful and productive collaboration with many creative, diligent and at the same time “gezellig” colleagues from different disciplines, Universities and countries. The research leading up to this thesis was conducted in Innsbruck, and I am still grateful for all the support given to me by the Dept. of Anesthesiology and Critical Care Medicine, Innsbruck Medical University, especially by Volker Wenzel, Arnulf Benzer, Guenther Putz, Gottfried Mitterschiffthaler and Christian Keller. Furthermore, I am grateful to Ingrid Haller, Lars Klimaschewski and Barbara Hausott for their help and support on our studies on Local-anesthetic-induced neurotoxicity. At the AMC, I would like to express my thanks to Ivo van Schaik and Camiel Verhamme at the Dept. of Neurology, who introduced me to the Neurophysiology of the Primary Sensory Neuron and Diabetic
Neuropathy, and thereby laid the groundwork for hopefully many interesting Regional Anesthesia projects to come. My thanks go out to Heidelinde Fiegl, Innsbruck Medical University, and Martin Widschwender, University College London, for introducing me to the fascinating world of Epigenetics.

Along my academic career, I have been fortunate to encounter colleagues who have influenced and inspired me. Among them, I would especially thank Peter Gerner, Jim Rathmell and Quinn Hogan for being such exemplary role models as Clinician Scientists and in the care they afford to their patients.

I am grateful to John McDonough and Juergen Osterbrink for giving me insight into the discipline of Nurse anesthesia, for our many creative and fruitful discussions, and the good collaboration on our mutual projects.

To my good friends Lukas Kirchmair and Josef Rieder (in alphabetical order), I am grateful for your friendship and for our many common steps in Research, in Anesthesia, and as friends.

Coming to Amsterdam also led to a wonderful collaboration with Susanne Picardi at the University Hospital in Heidelberg. My gratitude for our interesting mutual projects is linked to the hope that we will collaborate on many topics surrounding local anesthetics in the years to come.

My two “paranimfen”, Holger Baumann and Jan Eshuis, I am grateful for your support even from before the first day in Amsterdam, and that you will be at my side on this important day.

Any good anesthesia department needs a well-balanced mix of clinicians, clinician-scientists and scientists. I think that our Department of Anaesthesiology fits this description very very well and I am grateful to be working in such a supporting and supportive environment.

Moving to Amsterdam almost four years ago was, naturally, a life event for my family and me, but our arrival was facilitated and soothed by incountable colleagues and friends who helped, supported, listened, gave advice, and made us feel welcome. Hartelijk bedankt voor jullie steun en hulp.
The famous last words and words of gratitude belong to my family. Specifically, I would like to thank my parents for supporting me all my life and for affording me the liberty to pursue the study of my dreams, Medicine. Most importantly, I would love to thank you, Petra, for our wonderful life together, and you, Ferdinand and Leopold, for being such wonderful sons. Without you three (all being well soon to be four), this Thesis would not be here, and that is literally the case from front-page art to this very last page. And even if something was there, without you it would not make sense. Ich lieb Euch, sehr sehr.
Local anesthetics

New insights into risks and benefits

Philipp Lirk

Hierbij nodig ik u uit voor het bijwonen van de openbare verdediging van mijn proefschrift getiteld

Local anesthetics
New insights into risks and benefits

De verdediging vindt plaats woensdag 11 juni 2014 om 14:00 in de

Agnietenkapel
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