Local anesthetics: New insights into risks and benefits

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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
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...
Needle trauma and intraneural injection

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 an 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
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. 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. 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.

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. Our findings confirm and widen previous reports by Chan et al. 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. In contrast, the extraneural fluid reservoir injected in control animals was significantly decreased in volume after three hours.
Needle trauma and intraneural injection

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
considered feasible after 10-14 days.\textsuperscript{19} 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,\textsuperscript{20} 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.

\textit{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.\textsuperscript{21}

\textit{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.

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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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</thead>
<tbody>
<tr>
<td>Group B</td>
<td>6</td>
<td>164.5 ± 77.2</td>
<td>100</td>
<td>300</td>
</tr>
<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>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<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>
</tr>
<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>
<td>0.7 (0.2)</td>
<td>0.6 (0.3)</td>
<td>0.5 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<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>
</tr>
<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
Needle trauma and intraneural injection

Figure 2A

Figure 2B
Figure 3