Organ protection by the noble gas helium
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Chapter 2

Noble gases as cardio-protectants – translatability and mechanism

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ABSTRACT

Several noble gases, although classified as inert substances, exert in different experimental models tissue protection when applied before organ ischaemia as an early or late preconditioning stimulus, after ischemia as a postconditioning stimulus, or when given in combination before, during and/or after ischemia. A wide range of organs can be protected, in particular cardiac and neuronal tissue. We here summarize data on noble-gas induced cardio-protection, focussing on the underlying protective mechanisms. We will also look at translatability of experimental data to the clinical situation.
INTRODUCTION

The noble gases helium, neon, argon, krypton, xenon and radon are odourless, colourless, monatomic gases that are characterized by a filled outer shell of valence electrons, making them ‘inert’ or at least less capable of interaction with other compounds. However, some of these gases are already frequently used in medicine, e.g. helium is applied to patients with severe airway disease because of its very low density.\(^1\)\(^,\)\(^2\) Xenon has been shown to act at the N-methyl-D-aspartate receptor\(^3\) thereby inducing anaesthesia under normobaric conditions.\(^4\)

In recent years, several investigators demonstrated a tissue-protective effect of noble gases in different animal species as well as in humans.\(^5\)\(^-\)\(^8\) This protection was shown for various periods of ischemia-reperfusion, e.g. when the gas was applied before organ ischemia as an early or late preconditioning stimulus,\(^9\)\(^-\)\(^11\) after ischaemia as a postconditioning stimulus,\(^12\)\(^,\)\(^13\) or when given in combination before, during and/or after ischemia.\(^14\) A wide range of organs can be protected by these inert substances, in particular the heart and neuronal tissue.\(^6\)\(^,\)\(^8\)\(^,\)\(^15\) This article will summarize the current knowledge of noble-gas induced cardio-protection and will focus on the mechanisms of protection and a possible translatability to the clinical situation.

XENON

Cardioprotection by xenon

Anaesthetic properties of xenon have been described as early as 1951,\(^16\) and during the last two decades numerous studies evaluated molecular properties\(^8\) and clinical advantages\(^17\) of xenon as an anaesthetic agent. Within the on-going discussion on anesthesia-induced post-operative cognitive dysfunction in the elderly surgical patient, xenon might be advantageous compared to commonly used inhalational anesthetics,\(^18\)\(^,\)\(^19\) although this protective effect has been challenged by other investigators.\(^20\)

By far, most information on organ protection by noble gases comes from studies using xenon as an inhalational agent. Experimental studies clearly showed protection of the brain,\(^21\) spinal cord,\(^22\)\(^,\)\(^23\) kidney,\(^24\) heart\(^15\)\(^,\)\(^25\) and vascular endothelium\(^26\) against ischemia-reperfusion injury by xenon.

Applying the noble gas at the end of ischaemia and during the first minutes of reperfusion might also have protective effects leading to infarct size reduction by post-conditioning. In rabbits subjected to 30 min of coronary artery occlusion followed by 120 min of reperfusion, inhalation of xenon (70%) at the very end of regional myocardial ischemia and during the first 15 min of reperfusion (post-conditioning) reduced infarct size.\(^27\) Post-conditioning by sub-anaesthetic concentrations of xenon (20%) combined with mild hypothermia during early reperfusion also reduced myocardial damage in rats in vivo.\(^18\)
Much more information is available for myocardial pre-conditioning with xenon. Cardioprotection by xenon might be established if the gas is given as an early (within 2-3 h) or late (within 12-24 h) pre-conditioning stimulus before organ ischemia occurs: in rats subjected to 25 min of regional myocardial ischaemia followed by 2 h of reperfusion, xenon inhalation for 3 times 5 minutes before myocardial ischemia significantly reduced infarct size from 51% of the area at risk to 28%.\(^\text{31}\)

**Mechanisms of xenon induced cardioprotection**

There are numerous enzymes and cellular structures involved in mediating the organ protective effects of conditioning. Namely the Survivor Activating Factor Enhancement (SAFE) pathway and the Reperfusion Injury Salvage Kinase (RISK) pathway have been suggested to play significant roles in mediating tissue protection.\(^\text{29}\) The SAFE pathway is influenced by e.g. the Janus kinase (JAK), the signal transducer and activator of transcription (STAT) and the mitochondrial permeability transition pore (mPTP). The RISK pathway involves numerous intracellular mediators, like e.g. phosphatidylinositol 3 kinase (PI3K), protein kinase c (PKC), mitogen activated protein kinase (MAPK), glycogen synthase kinase 3B and extracellular regulated kinases (ERK).\(^\text{29}\) It is likely that these pathways, which have mainly been described for ischemic conditioning,\(^\text{30}\) also play a significant role in pharmacological conditioning by noble gases. Figure 1 summarizes the possible mechanisms of noble gas induced cardio protection.

**Mechanism of xenon early preconditioning**

The infarct size reduction by xenon pre-conditioning\(^\text{11}\) was completely blocked by infusion of a protein kinase C (PKC) inhibitor or a p38 mitogen-activated protein kinase (MAPK) inhibitor, demonstrating that these enzymes play a significant role in xenon induced pre-conditioning. Xenon significantly increased phosphorylation of the isoform PKC-ε, and this regulation was blocked by the PKC inhibitor but not by the MAPK inhibitor. These data show that p38 MAPK is located downstream of PKC in the signalling cascade of xenon-induced pre-conditioning. Further experiments revealed that p38 MAPK is directly activated by xenon (an effect again blocked by a PKC inhibitor) and that the downstream target of p38 MAPK, the MAPK-activated protein kinase 2 (MAPKAPK-2) is also phosphorylated after xenon preconditioning.\(^\text{25}\) Using immunofluorescence staining it was shown that xenon induced a translocation of PKC-ε from the cytosol to the membrane fraction of myocardial tissue. To summarize, xenon reduced infarct size by a PKC-ε and MAPK dependent mechanism whereby the PKC-ε dependent mechanism is mediated by a translocation of PKC-ε from the cytosol to the membrane fraction of the cardiomyocytes. Next to MAPKAPK-2, also the small heat shock protein (HSP) 27 plays an important role in the reorganization of the actin cytoskeleton network of the cell. HSP27 was phosphorylated and translocated to the particulate fraction of cell homogenates after xenon inhalation. Looking at the actin cytoskeleton, xenon increased the polymerization of F-actin fibers, and these fibers were co-localized with the phosphorylated pHSP27.\(^\text{25}\)
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Figure 1: Schematic diagram showing mechanisms underlying the protective effects of noble gases. This figure is a summary of the mechanisms that may contribute to organ protection by noble gases, mainly via the RISK pathway. Gas names are shown circles (He, Ar, Xe). Green arrows indicate an activating or up-regulatory effect, whereas red dots indicate a suppressive or down-regulatory effect.

PKC can be activated by translocation, by phosphorylation of a threonine or serine residue, mediated by the 3-phosphatidylinositol-dependent kinase-1 (PDK-1), as well as by free radical release induced by activation of the mitochondrial adenosine triphosphate dependent potassium channel (K_{ATP} channel). Inhibitors of both target pathways blocked xenon-induced infarct size reduction as well as PKC phosphorylation. Absence of PKC activation in the presence of a mitochondrial K_{ATP} channel blocker within the signalling cascade of xenon preconditioning makes it likely that opening of the K_{ATP} channel takes place upstream of PKC activation. PDK-1 was time-dependently activated by xenon before PKC-e was activated.
There are also other isoforms of PKC, namely PKC-δ and PKC-α. However, these isoforms were not involved in xenon preconditioning, suggesting an isoform specific activation of PKC-ε by xenon.³³ ³⁴ Similarly, other MAPK, like the extracellular signal-regulated kinase (ERK, p44/42 MAPK) and the stress-activated p54/46 MAPK (SAPK/JNK) are critically involved in cell differentiation, cell survival as well as in cellular apoptosis. While ERK is involved in xenon induced tissue protection and an inhibitor of ERK blocked the cardio-protective actions of xenon, blocking of JNK 1/2 and JNK3 had no effect on xenon-induced infarct size reduction.³³ These data suggest a specific regulation of different kinases by xenon pre-conditioning in the heart.

**Mechanism of xenon late preconditioning**

A second window of protection (late preconditioning, LPC) occurs 12-24 h after application of a preconditioning stimulus and lasts up to 72 h. Xenon-induced LPC reduced infarct size in rat hearts subjected to regional ischemia and reperfusion from 64% to 31% of the area at risk.³⁴ Co-administration of an inhibitor of cyclooxygenase 2 (COX-2) completely blocked this infarct size reduction, although there was no direct increase of COX-2 messenger RNA or COX-2 protein expression observed after xenon preconditioning.

**Endothelial protection by xenon**

An endothelial layer covers all vessels in the body, including coronary artery vessels. Cardioprotective effects of noble gases might therefore be mediated via changes within the endothelium. The endothelial cell surface is relatively non-adhesive for macromolecular structures, but this physiologic property might be significantly altered after ischaemia and reperfusion. Increased pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) will increase the expression of cell adhesion molecules (CAM) on the endothelial layer, thereby recruiting circulating leukocytes to the site of inflammation. In cultured human umbilical vein endothelial cells, TNF-α was applied to induce cell damage, leading to increased expression of intracellular (ICAM-1) and vascular (VCAM-1) adhesion molecules.²⁶ Pre-treatment of the cells with xenon as a pre-conditioning stimulus (3 times 5 minutes) reduced expression of messenger RNA and protein expression of ICAM-1 and VCAM-1, but had no effect on a third adhesion molecule, the E-selectin. In addition, xenon prevented the TNF-α induced increase in nuclear factor κB (NFκB) transcriptional activity. Xenon thus most likely confers preconditioning via an ICAM-1 and VCAM-1 mediated pathway that includes abrogation of NFκB activity.

Xenon blocks the calcium-dependent calcium influx in endothelial cells,³⁵ thereby affecting mechanisms regulating the calcium release-activated calcium channel of the plasma membrane. Taken together, these data show that xenon might significantly alter endothelial function, and therefore some of the organ protective properties of xenon in different organs might be mediated by changes within the endothelium.
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The organ-protective effects of xenon might also be caused by modulation of inflammatory reactions, which have been demonstrated in neuronal tissue as well as in blood. However, xenon had no effect on the inflammatory response to cardiopulmonary bypass (CPB) as measured by the pro-inflammatory interleukin (IL)-6 and the anti-inflammatory IL-10 cytokine levels in a rat model of CPB. These data confirmed previous findings from in-vitro experiments using human blood showing no modulation of the inflammatory response to CBP by xenon.

How might inert gases be able to induce cellular changes?

Although xenon – like other noble gases – is supposed to be inert, it is obviously from the above-mentioned experimental data that xenon is able to produce biological changes within different cells. Using x-ray crystallography studies it has been suggested that xenon may disrupt conformational changes of the proteins urate oxidase, an intracellular globular protein with large hydrophobic cavities, and Annexin V, a protein with a hydrophilic pore inside supposed to bind to cell membranes by a calcium-dependent action. Binding sites of xenon within the respective proteins are flexible gas cavities with no water inside.

A series of cell membrane receptors has been shown to be influenced by xenon, e.g. the N-methyl-D-aspartate receptors, the two-pore domain potassium channel TREK-1, the plasmalemmal adenosine triphosphate (ATP)-sensitive potassium (K\textsubscript{ATP}) channel, the nicotinic acetylcholine receptor as well as the 5-hydroxytryptamine type 3 receptor. Most of this knowledge comes from neuronal cells. Whether these cellular effects also play a role in myocardial protection by xenon remains unclear. However, the K\textsubscript{ATP} channel, at least the mitochondrial K\textsubscript{ATP} channel, has been demonstrated to be critically involved in preconditioning of the heart and xenon-induced cardioprotection might be mediated partly via this channel. Regarding the previously mentioned actions on cerebral N-methyl-D-aspartate receptors it has been shown that xenon competes with the co-agonist glycine at the glycine site of the N-methyl-D-aspartate receptor.

Translatability of xenon-related organ protection

Some experimental studies investigated protective effects of xenon in human tissue in vitro. Xenon limited cell loss and decreased caspase-3 activity in cultured human osteosarcoma cells, indicating an anti-apoptotic effect. In cultured renal tubular cells (HK-2 cells) subjected to oxygen and glucose deprivation, xenon was the only noble gas with cell-protective properties. In this cell type, subjected to hypoxia-hypothermia, xenon limited cell loss and promoted cell expression of HSP-70 and haemooxygenase-1. In an isolated cardiopulmonary bypass system filled with blood from healthy human volunteers, xenon had no effect on cellular markers of inflammation caused by the extracorporeal circulation. Fahlenkamp and colleagues compared the effects of xenon and sevoflurane anaesthesia on leucocyte function in surgical patients. Leucocyte subpopulations were not different, and phagocytosis and oxidative burst of granulocytes were reduced to the same extend in both groups. After ex vivo

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lipopolysaccharide stimulation, pro-inflammatory cytokine release was not affected by xenon. These data show that xenon has minimal influence on inflammatory activity in humans.

Xenon has virtually no direct influence on myocardial blood flow and global hemodynamics in healthy and diseased hearts. Therefore, use of xenon has been advocated in cardiac compromised patients and several studies have demonstrated intraoperative preservation of myocardial contractility and stable hemodynamics. Despite the huge amount of experimental studies on xenon conditioning of the heart and neuronal tissue, there are up to now no studies clearly translating the conditioning properties of xenon found in experimental studies to humans. One reason might be the limited availability of xenon, leading to very high costs of this noble gas. Another limitation for using xenon in clinical ischemia-reperfusion situation might have been the lack of suitable anesthesia machines to safely and cost-effectively deliver xenon. During the recent years some new machines have become available for xenon ventilation, and advances in recovery and recycling of xenon might further help to make use of this substance economically more valuable. Because xenon has much less hemodynamic side effects than routinely used volatile anesthetics like sevoflurane and desflurane, it might be advantageous to investigate in clinical studies on pre- and postconditioning possible beneficial effects of the noble gas.

Lockwood et al. performed a feasibility and safety study, applying xenon to patients undergoing coronary artery bypass grafting using extracorporeal cardiopulmonary bypass. Although the study was not randomized and does not allow firm conclusions on myocardial injury, there was a tendency towards reduced troponin release in patients receiving 20-50% xenon compared to patients ventilated without any xenon. In contrast, in a randomized trial in 30 patients undergoing cardiac surgery there was no difference in postoperative troponin release when using xenon compared to sevoflurane anesthesia. Bein and colleagues determined troponin and creatine kinase MB (CK-MB) release in high risk surgical patients subjected to aortic surgery under inhalational anesthesia with xenon or total intravenous anesthesia with propofol. The authors found very low levels of myocardial damage markers, with no significant differences between groups, although three patients in the propofol group had elevated troponin values in the postoperative period compared to zero patients in the xenon group. All clinical studies included only few patients, and therefore do not allow to draw any firm conclusion about cardio-protection of xenon in humans. A summary of the clinical studies concerning cardioprotection by xenon is given in table 1. Recently, a clinical trial finished inclusion of more than 500 patients, comparing the effects of xenon, propofol or sevoflurane based anaesthesia on postoperative myocardial damage after coronary artery bypass graft surgery. The results of this study should provide us a definitive answer to the question whether the beneficial cardio-protective effects of xenon observed in numerous animal studies are translatable to the clinical situation in humans.
Table 1: Table summarizing available data of noble gas induced protection in human tissue

<table>
<thead>
<tr>
<th>Injury</th>
<th>Noble Gas</th>
<th>Type</th>
<th>Method</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staurosporine, mitochondrial toxins</td>
<td>He, Ne, Ar, Kr, Xe (75%)</td>
<td>Osteosarcoma cells</td>
<td>Continuous</td>
<td>Xe and Ar limited cell loss and decreased caspase-3 activation</td>
<td>Spaggiari, 2013</td>
</tr>
<tr>
<td>Oxygen glucose deficiency</td>
<td>He, Ne, Ar, Kr, Xe (75%)</td>
<td>Renal tubular cells (HK-2)</td>
<td>Preconditioning</td>
<td>Xe protects against cell death, He is cytotoxic, others gases had no effect</td>
<td>Rizvi, 2010</td>
</tr>
<tr>
<td>None</td>
<td>Ar (50%)</td>
<td>Astroglial cells Microglial cells (BV-2)</td>
<td>Continuous</td>
<td>Ar enhanced ERK 1/2 activity in microglia via the upstream kinase MEK</td>
<td>Fahlenkamp, 2012</td>
</tr>
<tr>
<td>Oxygen glucose deficiency</td>
<td>Xe (80%)</td>
<td>Neuronal glial cells</td>
<td>Preconditioning</td>
<td>Xe limited cell loss via K-ATP channel activation</td>
<td>Bantel, 2009</td>
</tr>
<tr>
<td>Hypothermia-hypoxia</td>
<td>Xe (70%)</td>
<td>Renal tubular cells (HK-2)</td>
<td>Preconditioning</td>
<td>Xe limited cell loss and promoted cell expression of heat-shock protein 70 and haemeoxygenase-1</td>
<td>Zhao, 2013</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>He (79%)</td>
<td>Blood from healthy volunteers</td>
<td>Preconditioning</td>
<td>30 and 60 min of helium inhalation does not affect immune system function</td>
<td>Oei 2012</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>Xe (60%)</td>
<td>Blood from patients undergoing elective abdominal surgery</td>
<td>Continuous</td>
<td>Xe provides modest anti-inflammatory and no pro-inflammatory effect. ERK1/2 phosphorylation in leucocytes was reduced after 1 h of Xe anaesthesia</td>
<td>Fahlenkamp, 2014</td>
</tr>
<tr>
<td>Cardiopulmonary bypass</td>
<td>Xe (50%)</td>
<td>Blood from healthy volunteers</td>
<td>Continuous</td>
<td>Xe had no effect on CPB induced leucocyte and platelet activation after CPB</td>
<td>Saravanan, 2009</td>
</tr>
<tr>
<td>Cardiopulmonary bypass</td>
<td>Xe (70%)</td>
<td>Blood from healthy volunteers</td>
<td>Continuous</td>
<td>Xe had no effect on cytokine (IL-8, IL-10, TNF) and adhesion molecule expression L-selectin, CD-18, CD-11b) after CPB</td>
<td>Bedi, 2002</td>
</tr>
<tr>
<td>Forearm I/R 15 min</td>
<td>He (50%)</td>
<td>Human volunteers</td>
<td>Continuous</td>
<td>He had no effect on endothelium, but decreased expression of CD11b and ICAM on leukocytes ad CD42b and PSGL-1 on platelets</td>
<td>Lucchini, 2009</td>
</tr>
<tr>
<td>Forearm I/R 20 min</td>
<td>He (79%)</td>
<td>Human volunteers</td>
<td>Preconditioning</td>
<td>He protects posts ischemic endothelial function, Blocking eNOS did not abolish this effect</td>
<td>Smit, 2013</td>
</tr>
<tr>
<td>None</td>
<td>Xe (59%)</td>
<td>Healthy volunteers</td>
<td>Continuous</td>
<td>Xenon had minimal effects on coronary flow dynamics</td>
<td>Schaefer, 2011</td>
</tr>
<tr>
<td>Injury</td>
<td>Noble Gas</td>
<td>Type</td>
<td>Method</td>
<td>Outcome</td>
<td>Reference</td>
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<tr>
<td>None</td>
<td>Xe (65%)</td>
<td>Continuous</td>
<td>Injury type</td>
<td>Continuous Xe anesthesia has higher mean arterial blood pressure and better left ventricle ejection fraction</td>
<td>Baumert, 2008</td>
</tr>
<tr>
<td>Out-of-hospital cardiac arrest</td>
<td>Xe (47%)</td>
<td>Continuous</td>
<td>Injury type</td>
<td>Continuous Xe anesthesia has higher mean arterial blood pressure and better left ventricle ejection fraction</td>
<td>Baumert, 2008</td>
</tr>
<tr>
<td>CABG</td>
<td>Xe (45-50%)</td>
<td>Continuous</td>
<td>Injury type</td>
<td>Continuous Xe anesthesia is feasible and safe compared to total venous anesthesia</td>
<td>Stoppe, 2013</td>
</tr>
<tr>
<td>Aortic surgery</td>
<td>Xe (60%)</td>
<td>Continuous</td>
<td>Injury type</td>
<td>Continuous Xe anesthesia is feasible and safe compared to total venous anesthesia</td>
<td>Bein, 2008</td>
</tr>
<tr>
<td>CABG</td>
<td>Xe (20, 35, 50%)</td>
<td>Continuous</td>
<td>Injury type</td>
<td>Continuous Xe anesthesia is feasible and safe compared to total venous anesthesia</td>
<td>Lockwood, 2006</td>
</tr>
<tr>
<td>Intracardiac device implantation</td>
<td>Xe (60-65%)</td>
<td>Continuous</td>
<td>Injury type</td>
<td>Continuous Xe anesthesia is feasible and safe compared to total venous anesthesia</td>
<td>Baumert, 2005</td>
</tr>
</tbody>
</table>

In this table a summary of the available data of noble gases is human tissue or organs is presented. From left to right: Injury: injury type against which protection was induced; Noble gas: the type of gas used with used concentration in brackets. Type: type of patients or cell type; Method: type of stimulus used; Outcome: short summary of results; Reference: reference of original article.
In a feasibility and safety study in adults with out-of-hospital cardiac arrest, xenon (at least 40%) was given for 24 h during mild hypothermia, initiated at the moment of intensive care unit admission. Because these patients have both, cardiac as well as cerebral damage, xenon may be particularly advantageous in this patient population. The post-arrival incremental change of troponin T from baseline to 24, 48, and 72 h post resuscitation was significantly lower in patients treated with xenon + mild hypothermia compared to patients treated solely with mild hypothermia. There was no safety issue observed in these post-cardiac arrest patients ventilated with a xenon-oxygen mixture. Although these data indicate that xenon might be a suitable treatment addition in patients with cardiac arrest, we have to take into account that all clinical studies only determined surrogate parameters (e.g. enzyme release) for organ protection and that up to now no clinical outcome studies are available allowing a strong conclusion on xenon induced organ protection in a clinical ischemia-reperfusion situation. Regarding neuro-protection, several small studies were not able to demonstrate any positive effect of xenon on incidence of postoperative cognitive dysfunction in the elderly. A clinical challenge might be the experimental finding that any kind of pre- and postconditioning can negatively be influenced by co-morbidities like hypertension, diabetes, or simply senescence. Until today, no clinical data are available investigating a conditioning effect of xenon in co-morbid or elderly patients, although this group of patients would most likely be the most relevant population for organ protection.

**HELIUM**

In contrast to xenon, the noble gas helium has no anesthetic properties, and might therefore be used in awake patients subjected to ischemia-reperfusion situations, e.g. during percutaneous coronary interventions in patients with myocardial infarction.

No side effects of helium on global or regional hemodynamics have been described. Because helium has a low density, it reduces work of breathing and is therefore used in patients with airway diseases. Ventilators allowing application of helium by invasive and non-invasive ventilation strategies are available, and therefore helium might also be used during heart or vascular surgery, or in patients undergoing organ transplantation. Because this noble gas is much less expensive, helium might be an excellent alternative for organ protection in clinical ischemia-reperfusion situations.
Mechanisms of cardioprotection induced by helium

Preconditioning by helium
Besides xenon, also the non-anesthetic noble gas helium exerts profound organ-protective effects. Three times 5 minutes inhalation of 70% helium before 30 minutes of coronary artery occlusion followed by 3 h of reperfusion significantly reduced infarct size in rabbit hearts. These data show that the noble gas helium induces preconditioning of the heart.

Administration of a phosphatidylinositol-3-kinase (PI3K) antagonist, a mitogen/extracellular signal-related kinase 1 (MEK-1) inhibitor or an inhibitor of the 70-kDa ribosomal protein s6 kinase (p70s6kinase) abolished this helium-induced preconditioning, indicating a role for the so called “reperfusion injury salvage kinase (RISK) pathway” in helium-induced cardio-protection. As these pro-survival kinases inhibit glycogen synthase kinase 3b (GSK-3b) and apoptotic protein p53 degradation, Pagel et al. investigated whether inhibition of GSK-3b and p53 lowers the threshold of helium-induced protection. The authors could indeed demonstrate that with these pharmacological interventions the protection of helium could be facilitated, an effect that was also observed after morphine application. These data indicate an opioid-receptor mediated mechanism in helium-induced preconditioning which might play a significant role in patients subjected to ischemia-reperfusion, as morphine is a routinely used opioid analgesic applied in these clinical situations.

Opening of the mitochondrial permeability transition pore (mPTP) leads to mitochondrial dysfunction, and preconditioning might be beneficial by preserving cardiac mitochondrial function. The mPTP is postulated to be a possible end-effector for myocardial necrosis and apoptosis after ischaemia/reperfusion injury, and pro-survival kinases (PI3K, ERK1/2) and their downstream targets (endothelial nitric oxide synthase, p53, GSK-3β) all prevent opening of the mPTP. Application of a selective mPTP opener abolished helium-induced early preconditioning, indicating that helium eventually inhibits mPTP opening. Prolongation of post-ischemic acidosis has been shown to reduce myocardial infarct size, and correction of acidic pH after restoration of blood flow can cause mPTP opening, thereby inducing tissue damage. Transient alkalosis during early reperfusion abolished helium-induced cardio-protection, but the protection was restored by the mPTP inhibitor Cyclosporine A. These data suggest that helium prevents mPTP opening by maintaining intracellular acidosis during early reperfusion. The effect of radical oxygen species and the mitochondrial adenosine triphosphate-regulated potassium channel (K-ATP) in helium preconditioning was investigated by using the ROS scavengers N-Acetylcysteine and N-2-mercaptopropionyl glycine or the K-ATP-channel blocker 5-hydroxydeconate, respectively. All blockers completely abolished helium-induced cardioprotection, indicating radical oxygen scavengers and K-ATP channels mediate helium preconditioning. In addition, infusion of the non-selective nitric oxide synthase (NOS) inhibitor N-nitro-L-arginine methyl ester during helium preconditioning abolished cardioprotection, while in contrast infusion of a selective inducible NOS inhibitor or a selective neuronal
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NOS inhibitor had no effect, indicating that protection by helium is mediated by nitric oxide generated by endothelial NOS.78

Cardiac mitochondrial function can be further analysed by the rate of oxygen consumption of isolated mitochondria after administration of a complex 2 substrate (state 2), adenosine diphosphate (state 3), and after complete phosphorylation of adenosine diphosphate to adenosine triphosphate (state 4), respectively. Preconditioning by helium increased state 4 respiration, thereby reducing the respiratory control index and suggesting a mild mitochondrial uncoupling. This effect was blocked by a selective Ca2+ sensitive potassium channel blocker iberotoxin.79 More recent data show that the activation of Ca2+-sensitive potassium channels by helium preconditioning is mediated via protein kinase A.80

Interestingly, in contrast to the previously mentioned data on xenon conditioning, in helium-induced organ protection involvement of the various enzymes and kinases has only been shown by use of specific or unspecific blockers. Until now, no significant up- or down-regulation or phosphorylation of the different kinases or its products has been demonstrated.12

Postconditioning by helium
To gain more mechanistic insights into the protective effect of helium, the expression of genes involved in cell death and survival pathways was investigated after helium postconditioning in male rats subjected to ischaemia, ischemia/reperfusion (I/R), or I/R and 15 min of 70% helium at the onset of reperfusion.81 Helium postconditioning caused up-regulation of genes involved in necrosis (17 of 23) and pro-apoptosis (18 of 25). Simultaneously, 4 of 23 (necrosis) and 7 of 25 genes (pro-apoptosis) were down regulated. The majority of anti-apoptotic genes (9 of 11) and genes involved in autophagy (24 of 32) was up regulated after helium postconditioning. These data suggest that helium postconditioning at least partly abrogates execution of cell death programs thereby reducing myocardial infarct size.

Most of the above mentioned influences of helium on the heart were investigated in healthy myocardium. However, pathophysiological changes, e.g. hypertension, diabetes mellitus or ageing may block any conditioning effect. Helium conditioning is more difficult obtained in diseased animals: it is abolished in aged rats79,80 as well as in diabetic, Zucker obese rats.12 The combination of both, helium pre- with post-conditioning, was protective against infarct size development in spontaneous hypertensive rats, whereas each stimulus alone was not able to induce cardioprotection in the hypertensive rat heart.74

Effect of helium on caveolae and caveolins
Caveolae are cholesterol and sphingolipid enriched invaginations of the plasma membrane. Caveolins, the structural proteins essential for caveolae formation, are critically involved in anaesthetic-induced cardio-protection.82 Helium inhalation decreased Caveolin-1 and 3 expressions after 24 h.83 Buoyant Caveolin enriched fractions, indicative of increased Caveolin formation, supported the results showing lower Caveolin-1 and 3 levels in cytosolic and mi-
tochondrial fractions, while in contrast Caveolin-1/3 were accumulated in serum of mice 24 h after exposure to helium. These data indicate that Caveolin-1 and 3 are secreted into the blood after helium inhalation and support the hypothesis that circulating factors in the blood stream may be involved in inducing organ protection. This is in accordance with other means of conditioning.

Translatability of Helium-related organ protection

Similar to xenon, the tremendous bulk of data on helium induced organ protection in the heart was not definitively translated to the clinical situation.

Helium pre- and post-conditioning of the heart was investigated in patients undergoing coronary artery bypass grafting surgery. Patients were ventilated with a gas mixture of helium (70%) for 3 x 5 minutes before start of the cardiopulmonary bypass or at the moment of coronary reperfusion after declamping the aorta. In contrast to what would be expected from experimental data, neither helium pre- or postconditioning, nor a combination of pre- and postconditioning had any protective effect on post-operative troponin release.

Helium conditioning was investigated in healthy human volunteers subjected to forearm ischaemia and reperfusion. Using venous occlusion plethysmography to measure forearm blood flow responses to acetylcholine before and after 20 minutes of forearm ischaemia/reperfusion, we recently demonstrated that 3 times 5 minutes of 79% helium inhalation prevented post-ischaemic endothelial dysfunction. A similar protection was observed 24 hours after helium inhalation, demonstrating an early as well as a late endothelial preconditioning effect of helium in humans. Even after blocking endothelial NOS during helium inhalation the endothelial protection was maintained, meaning that the involvement of NOS found in previous animal studies could not be reconfirmed in humans.

Lucchinetti and colleagues applied 50% helium before, during and after ischaemia to healthy human volunteers and used post-ischaemic reactive hyperaemia to assess endothelial function before and after 15 min of forearm ischaemia. An increase of the pro-inflammatory marker CD11b and ICAM-1 on leukocytes and an attenuated expression of the pro-coagulant markers CD42b and PSGL-1 on platelets was observed. However, no changes in post-occlusive hyperaemic reaction were determined. While Smit et al. used 70% helium, Lucchinetti and colleagues applied 50% helium. These differences in helium concentration might have influenced the results, although in animal experiments helium as low as 30% induced myocardial protection (while 10% helium was not protective). The discrepancies of the results between the two studies might also be due to the different protocols of helium administration, as it was previously shown that continuous administration of a pharmacologic agent, e.g. a volatile anesthetic, does not induce cardioprotection while, in contrast, intermittent application with more than one cycle of inhalation of a volatile anesthetic did protect the human heart. In contrast to the study by Lucchinetti et al., another study in human volunteers did not show...
any effect of helium on the responsiveness of the innate and early adaptive immune system after 30 and 60 minutes helium inhalation.90

OTHER NOBLE GASES

Argon, Neon and Krypton
The noble gas argon has anaesthetic properties under hyperbaric conditions and is mainly investigated for its neuroprotective effects. After occlusion of the middle cerebral artery in rats, administration of 50% Argon reduced cerebral infarct size.91 In a model using cardiac arrest in rats, post-conditioning with argon 70% administered after resuscitation reduced histopathological damage of the neocortex and hippocampus. The mechanisms underlying this neuroprotective effect include up-regulation of ERK1/2 via MEK.92 Unlike neuroprotection by xenon, there seems no role for the N-Methyl-d-Aspartate (NMDA) receptor in argon induced organ protection.93 A recent study implicated a direct and concentration-dependent modulating effect of argon on enzymatic and thrombolytic effect of tissue plasminogen activator.93 A low concentration of argon (25%) blocked and high concentrations (75%) increased enzymatic and thrombolytic efficiency of tissue plasminogen activator. This might be interesting not only in patients with ischaemic stroke, but also in patients with myocardial infarction. With respect to cardioprotection, three cycles of 70% argon inhalation interspersed with washout periods reduced infarct size after regional myocardial ischaemia in rabbits.70 Until today, no clinical studies investigating organ protective effects of argon are available. In cultured human osteosarcoma cells, argon (as xenon) limited the cell loss induced by the broad-spectrum tyrosine kinase inhibitor staurosporine and several other mitochondrial toxins. In addition, argon inhibited the apoptotic activation of caspase-3.47 Neon reduced infarct size after regional myocardial ischemia in rabbits to the same extent as helium and argon.70 No Data on the underlying mechanisms are available. Neon and krypton had no neuroprotective effect in cortical neuronal cell cultures subjected to oxygen and glucose deprivation.94 Interestingly, in this model helium had detrimental effects on the cells and increased cell damage. In cultured human renal tubular cells (HK2) neon, argon and krypton showed no protection from cell injury provoked again by oxygen and glucose deprivation.95 No clinical studies are available investigating possible beneficial effects of neon or krypton in ischemia-reperfusion situations in humans.

CONCLUSION

Next to neuro- and cardioprotection, helium and xenon exert beneficial effects in the lung, kidney and liver. However, after summarizing the promising experimental data on tissue
protection, it remains to be proven whether this beneficial effect can be translated to the clinical situation. At this moment, there are not enough clinical data to allow any conclusion, although a lot of studies have been initiated with protocols published in trial registrations. The recently finished study on cardioprotection by xenon in coronary artery bypass graft surgery patients will answer the question whether xenon protects against ischemic cardiac damage after cardiac surgery. However, in this study, the most relevant group of patients, namely those patients with severely reduced myocardial function and very high risk for ischemic damage (e.g. combined coronary artery and valve surgery) were excluded. Thus future studies will have to include high-risk patients in order to show any beneficial effect on organ protection, thereby translating the promising experimental results from various models of organ damage to the clinical situation. A recent feasibility study already demonstrated that high risk patients after cardiac arrest and resuscitation could safely be ventilated using up to 50% of helium.96
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