BJP British Journal of Pharmacology

DOI:10.1111/bph.12994
www.brjpharmacol.org

Themed Section: Conditioning the Heart – Pathways to Translation

REVIEW

Noble gases as cardioprotectants – translatability and mechanism

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Received
29 August 2014
Revised
6 October 2014
Accepted
21 October 2014

Several noble gases, although classified as inert substances, exert a tissue-protective effect in different experimental models when applied before organ ischaemia as an early or late preconditioning stimulus, after ischaemia as a post-conditioning stimulus or when given in combination before, during and/or after ischaemia. A wide range of organs can be protected by these inert substances, in particular cardiac and neuronal tissue. In this review we summarize the data on noble gas-induced cardioprotection, focusing on the underlying protective mechanisms. We will also look at translatability of experimental data to the clinical situation.

LINKED ARTICLES

This article is part of a themed section on Conditioning the Heart – Pathways to Translation. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-8

Abbreviations

CPB, cardiopulmonary bypass; GSK, glycogen synthase kinase; HSP, heat shock protein; I/R, ischaemia/reperfusion; ICAM, intracellular cell adhesion molecules; K_{ATP}, ATP-dependent potassium; LPC, late preconditioning; MAPKAPK-2, MAPK-activated PK2; MEK, mitogen/ERK; mPTP, mitochondrial permeability transition pore; PDK-1, 3′phosphatidylinositol-dependent kinase-1; RISK, reperfusion injury salvage kinase; SAFE, survivor activating factor enhancement; VCAM, vascular cell adhesion molecule

Tables of Links

TARGETS	
Other protein targets[a]	**Enzymes**[f]
HSP70	Akt (PKB)
GRPCRs[b]	Caspase-3
5-HT_3 receptor	COX-2
Opioid receptors	Endothelial (e) NOS
Ligand-gated ion channels[c]	ERK
Nicotinic ACh receptors	GSK3β
NMDA receptors	Haem oxygenase 1
Ion channels[d]	Inducible (i) NOS
K_{ATP} channel	JNK
K_{Ca} channel	MAPKAPK-2
K_{2P}2.1 (TREK-1) channel	MEK1
Catalytic receptors[e]	Neuronal (n) NOS
CD11b	P38 MAPK
CD18	PDK1
	PI3K
	PKCα
	PKCδ
	PKCε
	Tissue plasminogen activator

LIGANDS	
ACh	LPS
ADP	Morphine
ATP	Propofol
Cyclosporin A	Sevoflurane
Desflurane	Staurosporine
Glycine	TNF-α
ICAM-1	Urate oxidase
L-NAME	VCAM-1

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d,,e,f]Alexander *et al.*, 2013a,b,c,d,e,f).

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, for example, helium is applied to patients with severe airway disease because of its very low density (Rodrigo *et al.*, 2002; Vorwerk and Coats, 2010). Xenon has been shown to act at the NMDA receptor (Franks *et al.*, 1998), thereby inducing anaesthesia under normobaric conditions (Rossaint *et al.*, 2003).

In recent years, several investigators demonstrated a tissue-protective effect of noble gases in different animal species as well as in humans (Preckel *et al.*, 2006; Oei *et al.*, 2010; Pagel, 2010; Deng *et al.*, 2014). This protection was shown for various periods of ischaemia reperfusion, for example, when the gas was applied before organ ischaemia as an early or late preconditioning (LPC) stimulus (Weber *et al.*, 2005a; Heinen *et al.*, 2008; Huhn *et al.*, 2009a), after ischaemia as a post-conditioning stimulus (Weber *et al.*, 2008a; Huhn *et al.*, 2009b) or when given in combination before, during and/or after ischaemia (Oei *et al.*, 2012b). A wide range of organs can be protected by these inert substances, in particular the heart and neuronal tissue (Preckel *et al.*, 2006; Oei *et al.*, 2010; Coburn *et al.*, 2012). This article will summarize the current knowledge on noble gas-induced cardioprotection 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 (Cullen and Gross, 1951), and during the last two decades numerous studies evaluated molecular properties (Preckel *et al.*, 2006) and the clinical benefits (Harris and Barnes, 2008) of xenon as an anaesthetic agent. With regard to the ongoing discussion on anaesthesia-induced postoperative cognitive dysfunction in elderly surgical patients, xenon might be advantageous compared with commonly used inhalational anaesthetics (Bronco *et al.*, 2010; Stuttmann *et al.*, 2010), although this protective effect has been challenged by other investigators (Rasmussen *et al.*, 2006; Höcker *et al.*, 2009).

By far the most information on organ protection by noble gases comes from studies using xenon as an inhalational agent. Experimental studies have clearly shown that xenon protects the brain (Limatola *et al.*, 2010), spinal cord

(Yamamoto *et al.*, 2010; Yang *et al.*, 2013), kidney (Ma *et al.*, 2009), heart (Weber *et al.*, 2005a,b) and vascular endothelium (Weber *et al.*, 2008c) from ischaemia reperfusion injury.

Applying the noble gas at the end of ischaemia and during the first minutes of reperfusion might also have protective effects leading to a reduction in infarct size 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 ischaemia and during the first 15 min of reperfusion (post-conditioning) reduced infarct size (Preckel *et al.*, 2000). Post-conditioning by sub-anaesthetic concentrations of xenon (20%) combined with mild hypothermia during early reperfusion also reduced myocardial damage in rats *in vivo* (Schwiebert *et al.*, 2010).

In comparison to its use for post-conditioning, much more information is available for myocardial preconditioning 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) preconditioning stimulus before organ ischaemia occurs: in rats subjected to 25 min of regional myocardial ischaemia followed by 2 h of reperfusion, xenon inhalation for three times 5 min before myocardial ischaemia significantly reduced infarct size from 51% of the area at risk to 28% (Weber *et al.*, 2005a).

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 (Hausenloy *et al.*, 2011). The SAFE pathway is influenced by, for example, the JAK, STAT pathways and the mitochondrial permeability transition pore (mPTP). The RISK pathway involves numerous intracellular mediators, such as PI3K, PKC, MAPK, glycogen synthase kinase 3β (GSK3β) and ERK (Hausenloy *et al.*, 2011). It is likely that these pathways, which have mainly been described for ischaemic conditioning (Przyklenk, 2013), also play a significant role in pharmacological conditioning by noble gases. Figure 1 summarizes the possible mechanisms of noble gas-induced cardioprotection.

Mechanism of xenon early preconditioning

The infarct size reduction by xenon preconditioning (Weber *et al.*, 2005a) was completely blocked by infusion of a PKC inhibitor or a p38 MAPK inhibitor, demonstrating that these enzymes play a significant role in xenon-induced preconditioning. Xenon significantly increased phosphorylation of the isoform PKCε, and this effect 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 preconditioning. 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 PK2 (MAPKAPK-2), is also phosphorylated after xenon preconditioning (Weber *et al.*, 2005b). Using immunofluorescence staining, it was shown that xenon induced a translocation of PKCε from the cytosol to the membrane fraction of myocardial tissue. To summarize, xenon reduced infarct size by a PKCε and MAPK-dependent mechanism whereby the PKCε-dependent mechanism is mediated by a translocation of PKCε from the cytosol to the membrane fraction of the cardiomyocytes.

In addition to MAPKAPK-2, the small heat shock protein (HSP) 27 also 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. From looking at the actin cytoskeleton, xenon was found to increase the polymerization of F-actin fibres, and these fibres were co-localized with the phosphorylated (p) HSP27 (Weber *et al.*, 2005b).

A central role for PKC in mediating the protective effects of different kinds of preconditioning has been confirmed in different animal species as well as in humans (Simkhovich *et al.*, 2013). 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 ATP-dependent potassium channel (K_{ATP} channel). Inhibitors of both target pathways blocked xenon-induced infarct size reduction as well as the phosphorylation of PKC (Weber *et al.*, 2006b). The 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 the opening of the K_{ATP} channel takes place upstream of PKC activation. PDK-1 was time-dependently activated by xenon before PKCε was activated.

There are also other isoforms of PKC, namely PKCδ and PKCα. However, these isoforms were not involved in xenon preconditioning, suggesting an isoform-specific activation of PKCε by xenon (Weber *et al.*, 2006a,b). Similarly, other MAPKs, such as ERK (p44/42 MAPK) and the stress-activated p54/46 MAPK, are critically involved in cell differentiation, cell survival as well as in cellular apoptosis. Although ERK is involved in xenon-induced tissue protection and an inhibitor of ERK blocked the cardioprotective actions of xenon, blocking of JNK1/2 and JNK3 had no effect on xenon-induced infarct size reduction (Weber *et al.*, 2006a). These data suggest a specific regulation of different kinases by xenon preconditioning in the heart (Weber *et al.*, 2006a).

Mechanism of xenon-induced late preconditioning LPC

A second window of protection (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 ischaemia and reperfusion from 64 to 31% of the area at risk (Weber *et al.*, 2008a). Co-administration of an inhibitor of COX-2 completely blocked this infarct size reduction, although there was no direct increase in COX-2 mRNA 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

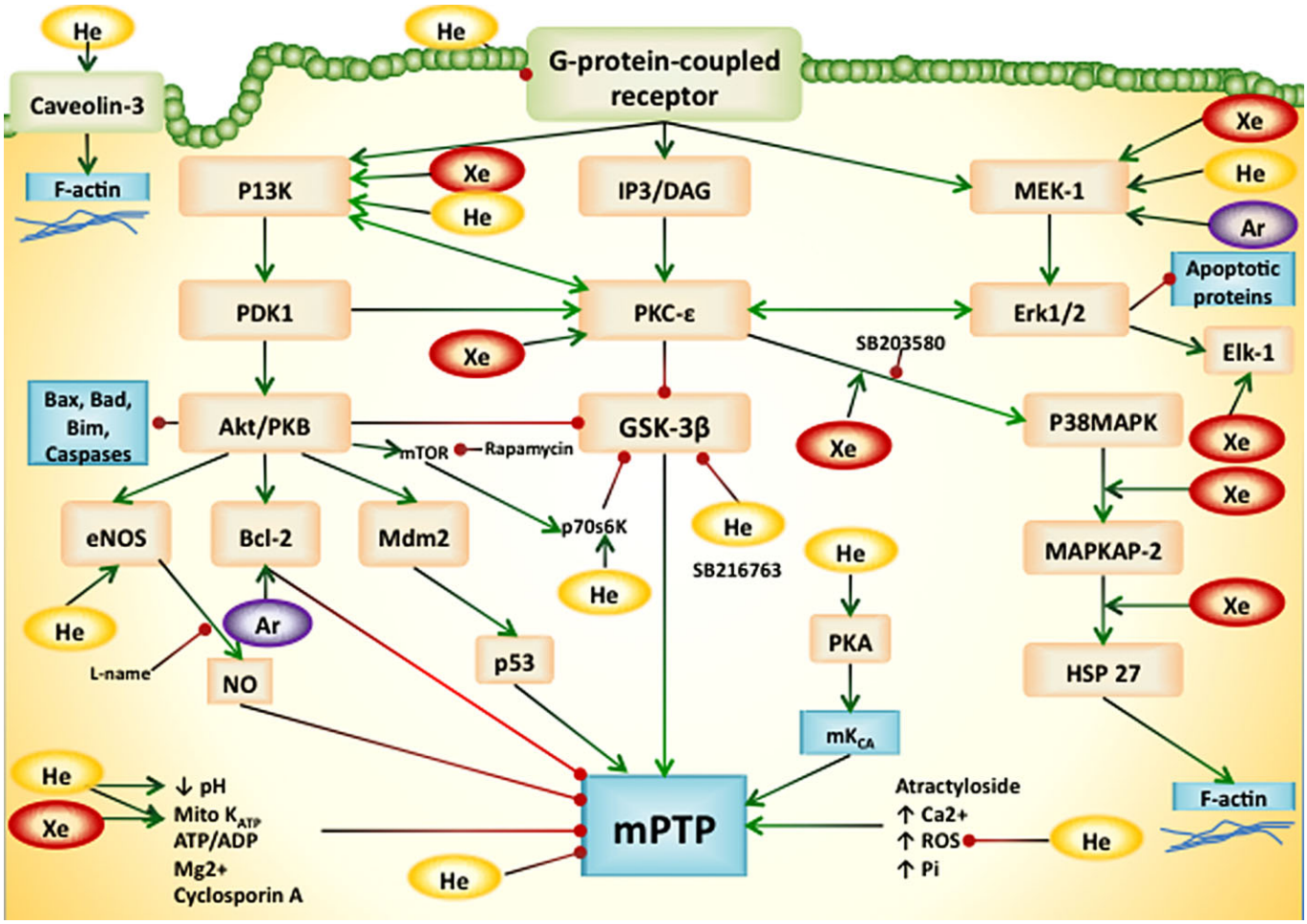

Figure 1

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 in circles (He, Ar, Xe). Green arrows indicate an activating or up-regulatory effect, whereas red dots indicate a suppressive or down-regulatory effect. Bcl-2, B-cell lymphoma-2; DAG, diacylglycerol; IP3, inositol triphosphate-3; Mdm2, murine double minute-2; mK_{Ca}, mitochondrial Ca^{2+}-sensitive potassium channel; p38MAPK, p38 MAPK; PDK-1, phosphoinositide-dependent PK-1; Pi, inorganic phosphate; ROS, reactive oxygen species.

gases might therefore be mediated via changes within the endothelium. The endothelial cell surface is relatively non-adhesive for macromolecular structures, but this physiological property might be significantly altered after ischaemia and reperfusion. Increased pro-inflammatory cytokines such as TNF-α will increase the expression of cell adhesion molecules on the endothelial layer, thereby recruiting circulating leukocytes to the site of inflammation. In cultured HUVEC, TNF-α was applied to induce cell damage, leading to increased expression of intracellular (ICAM-1) and vascular (VCAM-1) adhesion molecules (Weber *et al.*, 2008c). Pretreatment of the cells with xenon as a preconditioning stimulus (three times 5 min) reduced the expression of the mRNA and protein of ICAM-1 and VCAM-1, but had no effect on a third adhesion molecule, E-selectin. In addition, xenon prevented the TNF-α-induced increase in NF-κB transcriptional activity. Xenon thus most likely confers preconditioning via an ICAM-1- and VCAM-1-mediated pathway that includes inhibition of NF-κB activity.

Xenon blocks the calcium-dependent calcium influx in endothelial cells (Petzelt *et al.*, 1999), 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.

The organ-protective effects of xenon might also be caused by modulation of inflammatory reactions, which have been demonstrated in neuronal tissue (Fahlenkamp *et al.*, 2010) as well as in blood (de Rossi *et al.*, 2004). However, xenon had no effect on the inflammatory response to cardiopulmonary bypass (CPB) as measured by cytokine, pro-inflammatory IL-6 and anti-inflammatory IL-10, levels in a rat model of CPB (Clark *et al.*, 2005). These data confirmed previous findings from *in vitro* experiments using human blood showing that xenon had no effect on the inflammatory response to CBP (Bedi *et al.*, 2002).

How might inert gases be able to induce cellular changes?

Although xenon – like other noble gases – is supposed to be inert, it is obvious from the aforementioned 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 (Colloc'h *et al.*, 2007). 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, for example, the NMDA receptors (Franks *et al.*, 1998), the two-pore domain potassium channel TREK-1 (Gruss *et al.*, 2004), the plasmalemmal K_{ATP} channel (Bantel *et al.*, 2009), the nicotinic ACh receptor (Yamakura and Harris, 2000) as well as the 5-HT_3 receptor (Suzuki *et al.*, 2002). Most of this knowledge comes from neuronal cells. Whether these cellular effects also play a role in myocardial protection by xenon remain unclear. However, the K_{ATP} channel, at least the mitochondrial K_{ATP} channel, has been demonstrated to be critically involved in preconditioning of the heart (O'Rourke, 2004), and xenon-induced cardioprotection might be mediated partly via this channel. Regarding the previously mentioned actions on cerebral NMDA receptors, it has been shown that xenon competes with the co-agonist glycine at the glycine site of the NMDA receptor (Dickinson *et al.*, 2007).

Translatability of xenon-related organ protection

The protective effects of xenon have been investigated in human tissue *in vitro*. Xenon limited cell loss and decreased caspase-3 activity in cultured human osteosarcoma cells, indicating an anti-apoptotic effect (Spaggiari *et al.*, 2013). In cultured renal tubular cells (HK-2 cells) subjected to oxygen and glucose deprivation, xenon was the only noble gas with cell-protective properties (Rizvi *et al.*, 2010). In this cell type subjected to hypoxia–hypothermia, xenon limited cell loss and promoted cell expression of HSP70 and haemoxygenase-1 (Zhao *et al.*, 2013). In an isolated CPB system filled with blood from healthy human volunteers, xenon had no effect on cellular markers of inflammation caused by the extracorporeal circulation (Saravanan *et al.*, 2009). Fahlenkamp *et al.* (2014) compared the effects of xenon and sevoflurane anaesthesia on leukocyte function in surgical patients. Leukocyte subpopulations were not different, and phagocytosis and oxidative burst of granulocytes were reduced to the same extent in both groups (Fahlenkamp *et al.*, 2014). After *ex vivo* LPS stimulation, pro-inflammatory cytokine release was not affected by xenon. These data show that xenon has a minimal influence on inflammatory activity in humans.

Xenon has virtually no direct influence on myocardial blood flow and global haemodynamics in healthy and diseased hearts (Preckel *et al.*, 2002a,b). Therefore, the use of xenon has been advocated in cardiac-compromised patients (Baumert *et al.*, 2005; 2008; Bein *et al.*, 2005), and several studies have demonstrated intraoperative preservation of myocardial contractility and stable haemodynamics (Coburn *et al.*, 2005; Wappler *et al.*, 2007; Schaefer *et al.*, 2011). Despite the huge amount of experimental studies on xenon conditioning of the heart (Preckel *et al.*, 2006) and neuronal tissue (Deng *et al.*, 2014), there are, as yet, 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 the clinical ischaemia reperfusion situation might have been the lack of suitable anaesthesia machines to safely and cost-effectively deliver xenon. More recently, 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 viable (Dingley and Mason, 2007; Rawat and Dingley, 2010). Because xenon has many fewer haemodynamic side effects than routinely used volatile anaesthetics, such as sevoflurane and desflurane, it might be advantageous to investigate the possible beneficial effects of this noble gas in clinical studies on pre- and post-conditioning .

Lockwood *et al.* (2006) performed a feasibility and safety study, applying xenon to patients undergoing coronary artery bypass grafting using extracorporeal CPB. 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 with patients ventilated without any xenon. In contrast, in a randomized trial in 30 patients undergoing cardiac surgery there was no difference in post-operative troponin release when using xenon compared with sevoflurane anaesthesia (Stoppe *et al.*, 2013). Bein *et al.* (2005) determined troponin and creatine kinase-muscle/brain release in high-risk surgical patients subjected to aortic surgery under inhalational anaesthesia with xenon or total i.v. anaesthesia 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 post-operative period compared with zero patients in the xenon group. All clinical studies included only few patients and, therefore, do not allow any firm conclusion to be drawn about cardioprotection of xenon in humans. A summary of the clinical studies concerning cardioprotection by xenon is given in Table 1. Recently, a clinical trial (NCT01294163) finished, which included more than 500 patients and compared the effects of xenon, propofol or sevoflurane-based anaesthesia on post-operative myocardial damage after coronary artery bypass graft surgery (Hofland, 2014). The results of this study should provide us with a definitive answer to the question of whether the beneficial cardioprotective effects of xenon observed in numerous animal studies are translatable to the clinical situation in humans.

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 (Arola *et al.*, 2013), 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

Table 1

Table summarizing available data of noble gas induced protection in human tissue

Injury	Noble gas	Type	Method	Outcome	Reference
Staurosporine, mitochondrial toxins	He, Ne, Ar, Kr, Xe (75%)	Osteosarcoma cells	Continuous	Xe and Ar limited cell loss and decreased caspase-3 activation	Spaggiari *et al.*, 2013
Oxygen glucose deficiency	He, Ne, Ar, Kr, Xe (75%)	Renal tubular cells (HK-2)	Preconditioning	Xe protects against cell death, He is cytotoxic, other gases had no effect	Rizvi *et al.*, 2010
None	Ar (50%)	Astroglial cells Microglial cells (BV-2)	Continuous	Ar enhanced ERK1/2 activity in microglia via the upstream kinase MEK	Fahlenkamp *et al.*, 2012
Oxygen glucose deficiency	Xe (80%)	Neuronal glial cells	Preconditioning	Xe limited cell loss via K-ATP channel activation	Bantel *et al.*, 2009
Hypothermia–hypoxia	Xe (70%)	Renal tubular cells (HK-2)	Preconditioning Post-conditioning	Xe limited cell loss and promoted cell expression of HSP70 and haemoxygenase-1	Zhao *et al.*, 2013
LPS lipoteichoic acid anti-CD3/anti-CD28	He (79%)	Blood from healthy volunteers	Preconditioning	30 and 60 min of helium inhalation does not affect immune system function	Oei *et al.*, 2012a
LPS	Xe (60%)	Blood from patients undergoing elective abdominal surgery	Continuous	Xe provides modest anti-inflammatory and no pro-inflammatory effect. ERK1/2 phosphorylation in leukocytes was reduced after 1 h of Xe anaesthesia	Fahlenkamp *et al.*, 2014
Cardiopulmonary bypass	Xe (50%)	Blood from healthy volunteers	Continuous	Xe had no effect on CPB induced leukocyte and platelet activation after CPB	Saravanan *et al.*, 2009
Cardiopulmonary bypass	Xe (70%)	Blood from healthy volunteers	Continuous	Xe had no effect on cytokine (IL-8, IL-10, TNF) and adhesion molecule expression L-selectin, CD18, CD11b) after CPB	Bedi *et al.*, 2002
Forearm I/R 15 min	He (50%)	Human volunteers	Continuous	He had no effect on endothelium, but decreased expression of CD11b and ICAM on leukocytes ad CD42b and PSGL-1 on platelets	Lucchinetti *et al.*, 2009
Forearm I/R 20 min	He (79%)	Human volunteers	Preconditioning	He protects post-ischaemic endothelial function Blocking eNOS did not abolish this effect	Smit *et al.*, 2013
None	Xe (59%)	Healthy volunteers	Continuous	Xenon had minimal effects on coronary flow dynamics	Schaefer *et al.*, 2011
None	Xe (65%)	CAD patients undergoing non-cardiac surgery	Continuous	Xe anaesthesia has higher mean arterial blood pressure and better left ventricle ejection fraction	Baumert *et al.*, 2008
Out-of-hospital cardiac arrest	Xe (47%)	Out-of-hospital cardiac arrest patients	Post-conditioning	Xe + mild hypothermia is feasible and favourable with decreased troponin T release	Arola *et al.*, 2013
CABG	Xe (45–50%)	CABG	Continuous	Balanced xenon anaesthesia is feasible and safe	Stoppe *et al.*, 2013
Aortic surgery	Xe (60%)	Aortic surgery	Continuous	Xe does not improve haemodynamic parameters or troponin release compared with total venous anaesthesia	Bein *et al.*, 2008
CABG	Xe (20, 35, 50%)	CABG	Continuous	Xe was safely and efficiently delivered to CABG patients while on CPB	Lockwood *et al.*, 2006
None	Xe (60–65%)	Intracardiac device implantation	Continuous	Xe preserves mean arterial blood pressure and left ventricle ejection fraction compared with propofol	Baumert *et al.*, 2005

Injury, injury type against which protection was induced; noble gas, type and concentration of gas used; type, type of patients or cell type; method, type of stimulus used; outcome, short summary of results; reference, reference of original paper.
CABG, coronary artery bypass grafting; CAD, coronary artery disease.

lower in patients treated with xenon + mild hypothermia compared with 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 determine indirect 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 ischaemia reperfusion situation. With regard to neuroprotection, several small studies were not able to demonstrate any positive effect of xenon on incidence of post-operative cognitive dysfunction in the elderly (Rasmussen *et al.*, 2006; Coburn *et al.*, 2007; Höcker *et al.*, 2009; Cremer *et al.*, 2011). A clinical challenge might be the experimental finding that any kind of pre- and post-conditioning can be negatively influenced by co-morbidities such as hypertension (Oei *et al.*, 2012b), diabetes (Weber *et al.*, 2008b) or simply senescence (Heinen *et al.*, 2008). As yet, no clinical data are available demonstrating a conditioning effect of xenon in co-morbid or elderly patients, although this group of patients is likely to be the most pertinant population for organ protection.

Helium

In contrast to xenon, the noble gas helium has no anaesthetic properties, and might therefore be used in awake patients subjected to ischaemia reperfusion situations, for example, during percutaneous coronary interventions in patients with myocardial infarction.

Helium has not been found to have any side effects on global or regional haemodynamics. Because helium has a low density, it reduces the energy needed to breathe 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 than xenon, helium might be an excellent alternative for organ protection in clinical ischaemia reperfusion situations.

Mechanisms of cardioprotection induced by helium

Preconditioning by helium. Besides xenon, the non-anaesthetic noble gas helium also exerts profound organ-protective effects (Oei *et al.*, 2010). Three times 5 min inhalation of 70% helium before 30 min of coronary artery occlusion followed by 3 h of reperfusion significantly reduced infarct size in rabbit hearts (Pagel *et al.*, 2007). These data show that the noble gas helium induces preconditioning of the heart.

Administration of a PI3K antagonist, a mitogen/ERK 1 (MEK1) 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 RISK pathway in helium-induced cardioprotection. As these pro-survival kinases inhibit GSK3β and apoptotic protein p53 degradation, Pagel *et al.* (2008a) investigated whether inhibition of GSK3β 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 (Pagel *et al.*, 2009). These data indicate an opioid receptor-mediated mechanism in helium-induced preconditioning, which might play a significant role in patients subjected to ischaemia reperfusion as morphine is a routinely used opioid analgesic applied in these clinical situations.

Opening of the 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 (I/R) injury, and prosurvival kinases (PI3K, ERK1/2) and their downstream targets (endothelial NOS, p53, GSK3β) all prevent opening of the mPTP (Hausenloy *et al.*, 2009). Application of a selective mPTP opener abolished helium-induced early preconditioning (Pagel *et al.*, 2008a), indicating that helium eventually inhibits mPTP opening. Prolongation of post-ischaemic acidosis has been shown to reduce myocardial infarct size (Preckel *et al.*, 1998), and correction of acidic pH after restoration of blood flow can cause mPTP opening (Cohen *et al.*, 2007), thereby inducing tissue damage. Transient alkalosis during early reperfusion abolished helium-induced cardioprotection, but the protection was restored by the mPTP inhibitor cyclosporine A (Pagel and Krolikowski, 2009). These data suggest that helium prevents mPTP opening by maintaining intracellular acidosis during early reperfusion. The effect of radical oxygen species and the mitochondrial ATP-regulated potassium channel (K_{ATP}) in helium preconditioning was investigated using the reactive oxygen species scavengers N-acetylcysteine and N-2-mercaptopropionyl glycine or the K_{ATP} channel blocker 5-hydroxydeconate respectively (Pagel *et al.*, 2008b). All blockers completely abolished helium-induced cardioprotection indicating radical oxygen scavengers, and that K_{ATP} channels mediate helium preconditioning. In addition, infusion of the non-selective NOS inhibitor *N*-nitro-*L*-arginine methyl ester during helium preconditioning abolished cardioprotection, whereas infusion of a selective inducible NOS inhibitor or a selective neuronal NOS inhibitor had no effect, indicating that protection by helium is mediated by NO generated by endothelial NOS (Pagel *et al.*, 2008c).

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), ADP (state 3) and after complete phosphorylation of ADP to ATP (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 Ca^{2+}-sensitive potassium channel blocker iberiotoxin (Heinen *et al.*, 2008). More recent data show that the activation of Ca^{2+}-sensitive potassium channels by helium preconditioning is mediated via PKA (Huhn *et al.*, 2012).

Interestingly, in helium-induced organ protection the involvement of various enzymes and kinases has only been

shown by use of specific or non-specific blockers. Until now, for helium – in contrast to xenon – no significant up- or down-regulation or phosphorylation of the different kinases or its products has been demonstrated (Huhn *et al.*, 2009b).

Post-conditioning 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 post-conditioning in male rats subjected to ischaemia, I/R or I/R and 15 min of 70% helium at the onset of reperfusion (Oei *et al.*, 2013). Helium post-conditioning caused the 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) were up-regulated after helium post-conditioning. These data suggest that helium post-conditioning at least partly prevents the execution of cell death programmes, thereby reducing myocardial infarct size.

Most of the aforementioned influences of helium on the heart were investigated in healthy myocardium. However, pathophysiological changes, for example, hypertension, diabetes mellitus or ageing, may block any conditioning effect. Helium conditioning is more difficult to obtain in diseased animals: it is abolished in aged rats (Heinen *et al.*, 2008; Huhn *et al.*, 2012) as well as in diabetic, Zucker obese rats (Huhn *et al.*, 2009b). 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 (Oei *et al.*, 2012b).

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 cardioprotection (Horikawa *et al.*, 2008). Helium inhalation decreased caveolin-1 and 3 expressions after 24 h (Weber *et al.*, 2012). Buoyant caveolin-enriched fractions, indicative of increased caveolin formation, supported the results showing lower caveolin-1 and 3 levels in cytosolic and mitochondrial fractions, whereas 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 (Weber *et al.*, 2013) and support the hypothesis that circulating factors in the blood stream may be involved in inducing organ protection. This is in accord with other means of conditioning (Rassaf *et al.*, 2014).

Translatability of helium-related organ protection

Similar to xenon, the tremendous bulk of data on helium-induced organ protection in the heart (Oei *et al.*, 2010) 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 × 5 min before the start of the CPB 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 post-conditioning nor a combination of pre- and post-conditioning had any protective effect on post-operative troponin release (Smit *et al.*, 2012).

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 ACh before and after 20 min of forearm I/R, we recently demonstrated that three times 5 min of 79% helium inhalation prevented post-ischaemic endothelial dysfunction (Smit *et al.*, 2013). A similar protection was observed 24 h after helium inhalation, demonstrating an early as well as a late endothelial preconditioning effect of helium in humans *in vivo*. Even after blocking endothelial NOS during helium inhalation, the endothelial protection was maintained (Smit *et al.*, 2013), meaning that the involvement of NOS found in previous animal studies (Pagel *et al.*, 2008c) could not be reconfirmed in humans.

Lucchinetti *et al.* (2009) 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 in 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 the post-occlusive hyperaemic reaction were determined. Although Smit *et al.* (2013) 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) (Huhn *et al.*, 2009a). 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 pharmacological agent, for example, a volatile anaesthetic, does not induce cardioprotection, whereas intermitted application with more than one cycle of inhalation of a volatile anaesthetic did protect the human heart (Bein *et al.*, 2008; Frässdorf *et al.*, 2009). In contrast to the study by Lucchinetti *et al.* (2009), 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 min of helium inhalation (Oei *et al.*, 2012a).

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 (Ryang *et al.*, 2011). In a model using cardiac arrest in rats, post-conditioning with 70% argon administered after resuscitation reduced histopathological damage of the neocortex and hippocampus. The mechanism underlying this neuroprotective effect includes up-regulation of ERK1/2 via MEK (Fahlenkamp *et al.*, 2012). Unlike neuro-

protection by xenon, there seems no role for the NMDA receptor in argon-induced organ protection (Harris *et al.*, 2013). A recent study implicated a direct and concentration-dependent modulating effect of argon on enzymatic and thrombolytic effect of tissue plasminogen activator (David *et al.*, 2012): 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 (Pagel *et al.*, 2007). As yet, no clinical studies investigating organ-protective effects of argon are available. In human cultured osteosarcoma cells, argon (similar to 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 (Spaggiari *et al.*, 2013).

Neon reduced infarct size after regional myocardial ischaemia in rabbits to the same extent as helium and argon (Pagel *et al.*, 2007). 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 (Jawad *et al.*, 2009). Interestingly, in this model helium had detrimental effects on the cells and increased cell damage. In human cultured renal tubular cells (HK2) neon, argon and krypton showed no protection from cell injury provoked again by oxygen and glucose deprivation (Rizvi *et al.*, 2010). No clinical studies are available investigating possible beneficial effects of neon or krypton in ischaemia reperfusion situations in humans.

Conclusion

In addition 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 ischaemic 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 ischaemic damage (e.g. combined coronary artery and valve surgery), was 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 has already demonstrated that high-risk patients can be safely ventilated with up to 50% of helium after cardiac arrest and resuscitation (Brevoord *et al.*, 2012).

Conflict of interest

None of the authors has competing interests to be disclosed.

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