

Advances in molecular mechanism of cardioprotection induced by helium

Yi-ping Ding^{1, #}, Ju-yi Zhang^{2, #}, Dong-xia Feng³, Yan Kong^{1, *}, Zhuan Xu^{1, *}, Gang Chen²

1 Department of Neurology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, China

2 Department of Neurosurgery & Brain and Nerve Research Laboratory, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, China

3 Department of Scott & White Clinic-Temple, Temple, TX, USA

#These authors contributed equally to this work.

*Correspondence to: Yan Kong, M.D. or Zhuan Xu, M.D., kongyan@suda.edu.cn.

orcid: 0000-0001-6599-2416 (Yan Kong)

Abstract

Helium has been classified as a kind of inert gas that is not effortless to spark chemical reactions with other substances in the past decades. Nevertheless, the cognition of scientists has gradually changed accompanied with a variety of studies revealing the potential molecular mechanism underlying organ-protection induced by helium. Especially, as a non-anesthetic gas which is deficient of relevant cardiopulmonary side effects, helium conditioning is recognized as an emerging and promising approach to exert favorable effects by mimicking the cardioprotection of anesthetic gases or xenon. In this review we will summarize advances in the underlying biological mechanisms and clinical applicability with regards to the cardioprotective effects of helium.

Key words: helium; pre-conditioning; post-conditioning; cardioprotection; mechanism

doi: 10.4103/2045-9912.208519

How to cite this article: Ding YP, Zhang JY, Feng DX, Kong Y, Xu Z, Chen G. Advances in molecular mechanism of cardioprotection induced by helium. *Med Gas Res.* 2017;7(2):124-132.

Funding: This work was supported by Suzhou Key Medical Center (No. Szzx201501), grants from the National Natural Science Foundation of China (No. 81571115, 81422013, and 81471196), Scientific Department of Jiangsu Province (No. BL2014045), Suzhou Government (No. SZS201413, SYS201608, and LCZX201601), Jiangsu Province (No. 16KJB320008, BK20160347, QNRC2016730).

INTRODUCTION

Helium

Helium, a colorless, odorless, tasteless monatomic element, heads the noble gas series (neon, argon, krypton, xenon, and the radioactive element radon) in the periodic table.¹ As a member of noble gases, it has outer shells completely filled with electrons, making it almost completely inert and thus less capable of forming covalent bonds with other elements.^{2,3} At the same time, helium is described as a “non-immobilizer”, which represents a gas that cannot only induce anesthesia but also possess other behavioral effects.⁴ Recent years have witnessed an increased interest in experimental and clinical investigations of helium, and it has been convincingly shown that helium is capable of exerting significant biologic effects in diversified basic

experiments.⁵

Physical properties and its application

Helium is the second most abundant element in the known universe after hydrogen and has many unusual physical properties.⁶ For example, it is the lightest noble gas (molecular weight 4 g/mol) and its melting and boiling points are the lowest among all elements.⁷ Compared to oxygen (1.43 g/m³) and nitrogen (1.25 g/m³), helium has less density (0.179 g/m³).⁸ The absolute viscosity of helium is 201.8 mp (oxygen: 211.4 mp; ordinary air: 188.5 mp).^{9,10} The low density and high viscosity of helium just in line with the principle of Renault formula, this exactly is the cause that helium can be utilized to potentially benefit the problems associated with airway obstruction by reducing respiratory distress and improving respiratory efficiency.¹¹



Studies within the past decade have increasingly demonstrated heliox inhalation (a mixture of helium and oxygen) could be a potential lung protective strategy in the clinical settings such as bronchiolitis, acute asthma exacerbations and chronic obstructive pulmonary disease.⁶ Furthermore, the volume of helium molecules is extremely small so that the diffusion rate is 2.5 times that of nitrogen, and its water-insoluble properties indicate that paralysis and toxicity are not easily caused *via* blood circulation even in high pressure environments in deep water. So we often employ helium-oxygen mixed gas (rather than nitrogen) in the proper proportion to effectively prevent deep water anesthesia, avoid decompression and increase the depth of diving when a diver needs to stay in the deep sea for a long time.¹²

Chemical properties and its application

The increasing evidences indicate that inert gases especially xenon and some volatile anesthetics can exert organ protection through influencing certain signaling pathways in diverse basic experiments.¹³ As a member of an inert gas, whether helium also has biochemical properties and thus produces a similar protective effect has received extensive attention. Furthermore, compared with xenon and other volatile anesthetics, helium can be easily and safely administered.¹³ It is essentially devoid of anesthetic properties and many studies have already revealed a stable hemodynamic profile when clinically applied.¹⁴ These properties would make helium a perfect alternative for organ-protection in the case of reversible or irreversible ischemia/reperfusion (I/R) damage in clinical scenes when anesthesia is not requisite.¹⁵ Findings from numerous experimental studies have demonstrated that helium acts as a protective agent against tissue injury in a variety of organs such as brain, heart, liver, and kidney damage, and helium's cardioprotection has received most extensive attention.^{16,17} This review contains the existing knowledge about the cellular effects of helium, which may bring about new clinical strategies of salvage in myocardium I/R injury.

Myocardial I/R injury

Ischemic heart disease is the primary cause of morbidity because of its detrimental clinical consequences, such as acute myocardial infarction, heart failure, myocardial stunning and arrhythmias.^{18,19} Early reperfusion is the cornerstone in the treatment of myocardial damage but the flipside of the coin is reperfusion injury.²⁰ Studies in the past have unraveled that therapeutic intervention with the purpose of reducing reperfusion-induced injury is beneficial at the time of opening the obstructed vessel.²¹ Naturally, it results in the discovery of pre- and post-conditioning.

Cardioprotection induced by pre-conditioning covers two phases, an early phase (early pre-conditioning), lasting for 2–3 hours after the pre-conditioning stimulus,²² and a late phase (late pre-conditioning), reappearing 24 hours after the initial stimulus and lasting for 2–3 days.²³ What has been proved is that short episodes of ischemia in advance of an ischemic event (ischemic pre-conditioning) generate cardioprotection, reduce infarct size and ease cell damage.²⁴ Post-conditioning is achieved by conditioning cycles at the onset of reperfusion,²⁵ the effectiveness and availability making it a promising clinical approach to alleviate I/R injury as well.^{26,27} According to the available research findings, several pathways contribute to the survival of myocardial cells *via* helium pre- and post-conditioning are as follows.

HEALTHY ANIMAL MODELS

Pre-conditioning by helium

Early pre-conditioning

Reperfusion injury salvage kinase (RISK) pathway: In 2007, Pagel et al.²⁸ provided the first evidence that helium was able to exert protective effects by pre-conditioning in ischemic myocardium and the underlying mechanism involves the activation of the RISK pathway. The experiment showed that three 5-minute cycles of 70% helium significantly decreased infarct size among rabbits subjected to a 30-minute left anterior descending coronary artery occlusion and 3 hours reperfusion. Application of Wortmannin, PD 098059, and rapamycin, the selective inhibitors of phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase 1/2 (Erk1/2), and 70-kDa ribosomal protein s6 kinase (p70s6K) were capable to abolish the cardioprotection induced by a brief, intermittent administration of helium. It further demonstrated that atractyloside, a selective opener of mitochondrial permeability transition pore (mPTP), could counteract the protection induced by helium pre-conditioning. It means that RISK pathway and its proposed putative mPTP end-effector have been intensely implicated in reduction of cell death during reperfusion.

Previous studies have revealed that activated pro-survival kinases from the RISK pathway regulate the transition state of the mPTP by inhibiting the activity of glycogen synthase kinase-3 β (GSK-3 β), preventing the degradation of apoptotic protein p53 and favorably affecting pro- *versus* anti-apoptotic protein balance.²⁹ The GSK-3 β or p53 inhibition-mediated protection effects occur in the downstream from PI3K.^{30,31} Employing SB 216763 and pifithrin- α , the selective inhibitors of GSK-3 β and p53 respectively, lowered the threshold of helium pre-conditioning *in vivo* through an mPTP-dependent mechanism. Pagel et al.³² therefore demonstrated that the inhibition of GSK-3 β and p53 favorably modulating mPTP and producing cardioprotection *via*



RISK pathway in the helium-induced pre-conditioning.

G protein-coupled receptor ligands including $\delta 1$ -opioid, adenosine, and bradykinin have been shown to activate pro-survival signaling and play significant roles in classical ischemic pre- and post-conditioning.³³ Pagel and co-workers³⁴ tested the hypotheses that morphine, a routinely applied opioid receptor in clinical practice, lowers the threshold of cardioprotection produced by helium in rabbits as well.

Mild intracellular acidosis: Persistent intracellular acidosis was demonstrated to reduce myocardial damage by inhibiting mPTP formation during early reperfusion.^{35,36} Cohen et al.³⁷ proved that transient maintenance of myocardial acidosis contributed to myocardium protective action during ischemic post-conditioning by ultimately maintaining the close state of mPTP. We can accordingly make assumptions based on the discoveries that cardioprotection induced by ischemic post-conditioning is dependent on not only the activation of pro-survival signaling kinase pathways but also the preservation of intracellular acidosis during early reperfusion. In order to test the hypothesis that intracellular pH is involved in helium pre-conditioning during early reperfusion, Pagel et al.³⁸ testified the decreases in myocardial infarct size induced by intermittent, repetitive exposures to helium were thoroughly abolished by transient metabolic alkalosis during early reperfusion. And the mPTP inhibitor cyclosporin A restored helium-induced reductions in infarct size in the presence of alkalosis. Current observations have indicated that helium pre-conditioning may protect the myocardium against ischemic injury by maintaining modest intracellular acidosis, preserving mPTP in its closed conformation and thereby attenuating myocardial necrosis.

Potassium channel: Previous investigation abundantly certified that reactive oxygen species (ROS) and mitochondrial adenosine triphosphate potassium (K_{ATP}) channels play complementary roles during ischemic or anesthetic pre-conditioning,^{39,40} but the roles of ROS and mitochondrial K_{ATP} channels in the process of helium preconditioning remain to be defined. Paul et al.⁴¹ verified that pretreatment with ROS scavengers N-acetylcysteine (NAC) and N-2 mercaptopropionyl glycine (2-MPG) abolished helium-induced cardioprotection, suggesting that ROS participated in helium pre-conditioning *in vivo*. The research further indicated that the pretreatment with K_{ATP} channels blocker 5-hydroxydecanoate (5-HD) completely abolished decreases in myocardial infarct size produced by helium, implicating the underlying function of mitochondrial K_{ATP} channels in this process as well.⁴¹

In addition to activation of K_{ATP} channels, another kind of K^+ channels: Ca^{2+} -sensitive potassium (K_{Ca}) channels (mK_{Ca}) seems to be complicated in the pre-conditioning. Studies showed helium-induced pre-conditioning reduced

infarct size accompanied with a significant decrease in the mitochondrial respiratory control index (state3/state4) by increasing state4 respiration. The K-channel antagonist iberiotoxin not only completely eliminates helium-induced infarct size reduction but also reduces helium-induced respiratory control index. From the data we conclude that the cardioprotection helium provided is associated with the activation of the mK_{Ca} channel and mild mitochondrial uncoupling.¹⁸ Recent data further points out: the activation of mK_{Ca} channel is mediated by protein kinase-A (PKA) in helium-induced pre-conditioning.⁴²

Endothelial nitric oxide synthase (eNOS)-nitric oxide (NO): To our knowledge, eNOS acts as a downstream target of PI3K and AKT (protein kinase B), which can increase the formation of NO by phosphorylating Ser residues.^{43,44} As a major participant, NO plays a pivotal role *via* promoting the translocation of ϵ -isoform protein kinase C (PKC- ϵ) and direct activation of K_{ATP} in ischemia-induced or volatile anesthetic-induced cardioprotection.^{45,46} Paul et al.⁴⁰ further provided the evidence that NO is involved in the helium-induced pre-conditioning and helium directly increases the production of NO in a NOS-dependent manner, rather than rely on I/R injury. Pretreatment with non-selective NOS inhibitor, N-nitro-L-arginine methyl ester (L-NAME), reduced the infarct size induced by helium. Neither inducible NOS (iNOS) antagonist aminoguanidine hydrochloride (AG) nor neuronal NOS (nNOS) inhibitor 7-nitroindazole (7-NI) had any effect on helium-induced cardioprotection. These studies suggest that eNOS can mediate helium pre-conditioning in rabbits but iNOS or nNOS cannot do it.

Late pre-conditioning

Huhn et al.⁴⁷ demonstrated for the first time that helium can induce late pre-conditioning and reduce infarct size by approximately 40% compared with non-preconditioned myocardium. Late pre-conditioning was performed by administering 70%, 50%, 30% and 10% helium respectively for 15 minutes during 24 hours before I/R. The results showed that 70%, 50% and 30% helium significantly reduced infarct size from 55% in controls to 40%, 34%, and 37%. In contrast, no reduction in infarct size was observed at 10% helium. In other words, cardioprotection is maximal with administration of one cycle of 30% helium. Nevertheless, repetitive 30% helium inhalation subsequently 2–3 days before I/R did not further enhance the cardioprotective effects. The results showed that helium-mediated cardioprotection had a dose-dependent effect but not a time-dependent effect. The cyclooxygenase-2 (COX-2) inhibitor NS-398 completely eliminated 30% helium-mediated protection. It elucidated COX-2 is involved in helium-mediated late pre-conditioning.



Noteworthy, the above experimental studies simply indicated diversified kinases and their targets participated in helium-induced pre-conditioning by using their specific or non-specific blocking agents, but the actual expression and activity of these kinases remain to be measured and proved.⁴⁸ How helium might affect these pro-survival kinases and enzymes and thus mediate cardioprotection is yet completely unknown.¹

Post-conditioning by helium

Altered gene expression

Helium post-conditioning is the most clinically relevant and feasible form of conditioning, the investigation of which has become an area of scientist interest because ischemic events often occur upon arrival of the patient in the hospital.^{49,50} In order to get more detailed mechanistic insights into the damage-ameliorating effects helium-induced during I/R, Oei et al.⁵¹ investigated helium post-conditioning by assessing cell damage and exploring the differential expression patterns of genes related to apoptosis, necrosis and autophagy following ischemia. The results revealed that 15 minutes of helium post-conditioning reduced the extent of I/R-induced cell damage and the beneficial effect was not observed under circumstance of 5 and 30 minutes of helium post-conditioning. Administration by 15 minutes of helium post-conditioning caused predominant up-regulation of genes involved in autophagy and inhibition of genes involved in apoptosis in comparison with I/R alone. It turned out that the reduction of cell damage that is at least partly induced by helium post-conditioning is mediated by the selective expression of genes that regulate programmed cell death.

Immune system

The underlying mechanisms of reperfusion-induced myocardial cell dysfunction are oxidative stress and an inflammatory burst.^{52,53} The innate immune system in I/R injury is a double-edged sword as a severe inflammatory burst is detrimental to the survival of cells but the inhibition of the innate immune system is accompanied with adverse consequences after myocardial infarction at the same time.^{54,55} Several processes occur during early reperfusion such as leukocyte activation and recruitment,⁵⁶ cytokine and reactive oxygen species burst, and endothelial dysfunction are capable to influence cell viability.⁵⁷

The influence of helium post-conditioning on infarct size and the I/R-induced immune response were explored by assessing the levels of protein and mRNA of pro-inflammatory cytokines. Rats inhaled 15, 30, or 60 minutes of 70% helium during reperfusion respectively. The results elucidated that 15 minutes of helium inhalation observ-

ably decrease infarct size from 43% to 21%, whereas 30 or 60 minutes of helium inhalation can hardly receive the similar profitable consequences. The protein levels of cytokine-induced neutrophil chemoattractant (CINC-3) and interleukin-1 beta (IL-1 β) cytokine-induced were increased when employing 30 or 60 minutes of helium compared to control. The mRNA levels of CINC-3, IL-1 β , interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF- α) were all increased when exposed to 30 minutes of helium in the myocardial tissue not directly subjected to I/R. Above findings turned out the effectiveness of the helium post-conditioning was intimately dependent on the duration of helium administration.⁵⁸ It is not clear that whether the higher levels of inflammatory cytokines is related to helium conditioning or just the causative of nature.

Caveolins

Caveolae, as the small flask-like invaginations of the cellular membrane, has been proven to be a subset of lipid rafts.³³ And caveolins are structural proteins essential for the formation of caveolae. Caveolins contain three isoforms, including Cav-1, -2, -3, and a scaffolding domain (CSD), which plays a pivotal role in the regulation and localization of signaling molecules.⁵⁹ The CSD is able to combined with several triggers, mediators of cardioprotective pathways known as RISK pathway proteins, such as PI3K, AKT, PKC isoforms, and ERK1/2.⁶⁰ Caveolins are involved in mPTP transitioning through the binding of signaling molecules to the CSD.⁶¹ The three isoforms all are found in myocardial tissue⁶² and cardiac myocyte-specific over-expression of Cav-3 lead to the upregulation of survival kinases and therefore produced protection against I/R injury.⁶³

In recent research, Flick et al.⁶⁴ examined the role of the caveolin-associated RISK pathway in helium post-conditioning-induced cardioprotection in rat heart so as to explore the possible underlying mechanism. The findings indicated that: 1) changes in Cav-1 and Cav-3 localization only after 15 minutes of helium post-conditioning and no difference after 5 or 30 minutes; 2) 15 minutes of helium post-conditioning significantly increased accumulation of Cav-1 and Cav-3 in the membrane fraction of ischemic cardiac tissue but no difference in the mitochondrial fractions; 3) The serum analysis did not present any differences in the serum for Cav-1 levels, whereas Cav-3 showed increased amounts within 15 minutes of helium post-conditioning; 4) 15 minutes of helium post-conditioning activates RISK pathway kinases ERK1/2 and AKT. In conclusion the study points out that: 1) helium post-conditioning seems to induce protective effects shortly after the onset of reperfusion; 2) caveolins and RISK pathway might be crucially involved in helium post-conditioning mediated cardioprotection; 3)



increased Cav-1 and -3 in the membrane fraction as well as the serum might indicate involvement of mitochondrial signaling.⁶⁴ The results are contrast to another study that helium inhalation decreased caveolin-1 and caveolin-3 expressions after 24 hours in mice heart.⁶⁵ One recent research suggested helium treatment induced secretion of Cav-1 and -3 into the serum,⁶⁶ which is consistent with increased Cav-3 levels

in the serum of current *in vivo* model in rats. Even though above studies employed different species and time periods, the consequences illuminated that secreted caveolin might potentially produce crucial protective effect on account of its availability in the whole body.

The experimental data for helium-induced cardioprotection are summarized in **Table 1**.

Table 1: Summary of molecular mechanism of helium-induced pre-conditioning (PC) and post-conditioning (postC)

Type of protection	Animals	Mechanism	Reference
EPC	Rabbits	Inhibition of PI3K, Erk1/2, p70s6K and the opener of mPTP blocked cardioprotection	Pagel et al. ²⁸
	Rabbits	Inhibitors of GSK-3 β and p53 lowered the threshold through a mPTP dependent mechanism	Pagel et al. ³²
	Rabbits	ROS scavengers and K _{ATP} channels blockers abolished cardioprotection	Pagel et al. ⁴¹
	Rabbits	Morphine lowered the threshold of helium preconditioning	Pagel et al. ³⁴
	Rabbits	Cardioprotection was abolished by transient alkalosis, restored by CsA	Pagel et al. ³⁸
	Rabbits	Cardioprotection was abolished by inhibitor of eNOS but not inhibitor of iNOS or nNOS	Pagel et al. ⁴⁰
	Rats	He5 reduced infarct size, abolished by PKA blocker	Huhn et al. ⁴²
LPC	Rabbits	COX-2 inhibitors abolished helium-induced cardioprotection	Huhn et al. ⁴⁷
PostC	Rats	He15 reduced cell damage, but He5, H30 can not; He15 changed the expression of genes involved in cell pathways	Oei et al. ⁵¹
	Rats	He15 reduced infarct size, H30 or He60 increased protein levels of CINC-3 and IL-1 β ; He30 increased the mRNA levels of CINC-3, IL-1, IL-6, TNF- α	Oei et al. ⁵⁸
	Rats	He15 increased the secretion of Cav-3 and the activation of Erk1/2 and AKT/PKB	Flick et al. ⁶⁴

Note: EPC: Early PC; LPC: late PC; PI3K: phosphatidylinositol 3-kinase; Erk1/2: extracellular signal-regulated kinase 1/2; p70s6k: 70-kDa ribosomal protein s6 kinase; GSK-3 β : glycogen synthase 3 β ; p53: tumor protein 53; ROS: reactive oxygen species; K_{ATP}: mitochondrial adenosine triphosphate potassium channel; CsA: cyclosporin A; eNOS: endothelial nitric oxide synthase; iNOS: inducible nitric oxide synthase; nNOS: neuronal nitric oxide synthase; COX-2: cyclooxygenase-2; He(5/15/30/60): (5/15/30/60) minutes of helium inhalation; CINC-3: cytokine-induced neutrophil chemoattractant; IL-1 β : interleukin-1 β ; IL-6: interleukin-6; TNF- α : tumor necrosis factor- α ; Cav-3: caveolin-3; AKT/PKB: protein kinase B; PKA: protein kinase-A.

Disease animal models

All of above studies were carried out in healthy animals, whereas a majority of patients with heart disease are aged or suffer from multiple co-existing chronic diseases such as hypertension and diabetes. The research in diseased animals is limited but still provides reliable guidance for us to convert basic experiments into clinical applications, the details are as follows.

Healthy Wistar and spontaneous hypertensive rats are both subjected to 25 minutes ischemia followed by 120 minutes reperfusion. The two kinds of rats are subjected to 70% helium inhalation for 15 minutes after index ischemia (PostC), or together with 15 minutes of helium 24 hours prior to ischemia (LPC + PostC), or a triple intervention with the addition of three repetitive, short cycles of 5 minutes helium inhalation before ischemia (EPC + LPC + PostC) respectively. We can conclude from the results that a triple intervention of helium conditioning could protect hypertensive myocardium against I/R injury, whereas a single intervention could not. The consequences showed that the existence of a “threshold” in hypertensive cardiac

muscle, in which the combination of helium stimuli provide a more intense stimulus than each stimulus alone. No increased phosphorylation of GSK- β or PKC- ϵ were observed in these groups exposed to helium conditioning, indicating that helium-induced conditioning is not involved in GSK- β or PKC- ϵ pathway in diseased animals.⁴⁹

The aging or diseased myocardium can not be protected by a single stimulus of helium conditioning was certified in the aged Wistar rats or pre-diabetic obese Zucker rats. The protective capability of helium pre-conditioning is entirely abolished in the obese Zucker rat, a extensively used animal model for pre-diabetic conditions of type 2 diabetes.⁵⁰ An effect of helium on Erk1/2 and AKT phosphorylation was not detected and a depressed activity of GSK-3 β was discovered in Zucker lean rats. Even through strengthening the stimulus of pre-conditioning could not overcome the threshold of reducing infarct size in the pre-diabetic heart. This may showed that only a combination of different conditioning stimuli at various time points could exert protection function in diseased myocardium. From previous data we can concluded that helium could induce



mitochondrial uncoupling and generate pre-conditioning *via* the activation of mK_{Ca} channel. Nevertheless, above effects are eliminated in the senescent heart.⁴² The damage at the level of the mK_{Ca} channel or its upstream signaling molecules may explain the aging-related elimination of helium-induced pre-conditioning. Early research further indicated that the regulation of adenylyl cyclase/PKA was a possible potential mechanism for the age-dependent loss of helium-induced cardioprotection.⁴²

Clinical applications

The underlying mechanisms of cardioprotection helium-induced have been investigated extensively in animal experiments. However, mechanistic data from human studies are scarce.

In the latest clinical research, 125 patients undergoing coronary artery bypass grafting (CABG) surgery were allocated randomizedly to several groups include helium pre-conditioning *via* three cycles of helium inhalation for 5 minutes and subsequent 5 minutes inhalation of oxygen-enriched air, helium post-conditioning group receiving 15 minutes of helium inhalation before release of the aortic cross clamp, or the combination of both. The investigations indicated that the inability of helium conditioning to produce cardioprotection and no any statistically significant difference was observed with respect to the activation of p38 mitogen activated protein kinase (p38 MAPK), ERK 1/2 or levels of heat shock protein 27 (HSP27) and PKC- ϵ in human heart induced by helium pre-conditioning, post-conditioning or the combination of both. The result was in contradiction with the anterior experiments we demonstrated. Further more, helium pre- and post-conditioning did not affect postoperative troponin release at various time points postoperatively in patients undergoing CABG surgery.⁶⁷

In an earlier study, the patients subjected to similar CABG surgery were administered helium inhalation (79%) for 5 minutes once for three sections prior to the start of cardiopulmonary bypass (He-Pre) or at the time when the perfusion was initiated (He-PostC). The result showed He-Pre or He-Post alone, and even the combination of both did not generate beneficial effects on the level of postoperatively troponin release.⁶⁸

In one recent study investigating whether helium breathing in healthy volunteers affects the response capability of the human immune system in whole blood *ex vivo*, healthy male volunteers were administered 30 minutes heliox (79% helium and 21% O_2) or ordinary air respectively. Blood was withdrawn at various time points after helium breathing and subsequently received incubation with lipopolysaccharide (LPS), lipoteichoic acid (LTA), T-cell stimuli anti-CD3/anti-CD28 (TCS) or RPMI (as control) for different duration including 0, 2, 4 and 24 hours. The pro-inflammatory

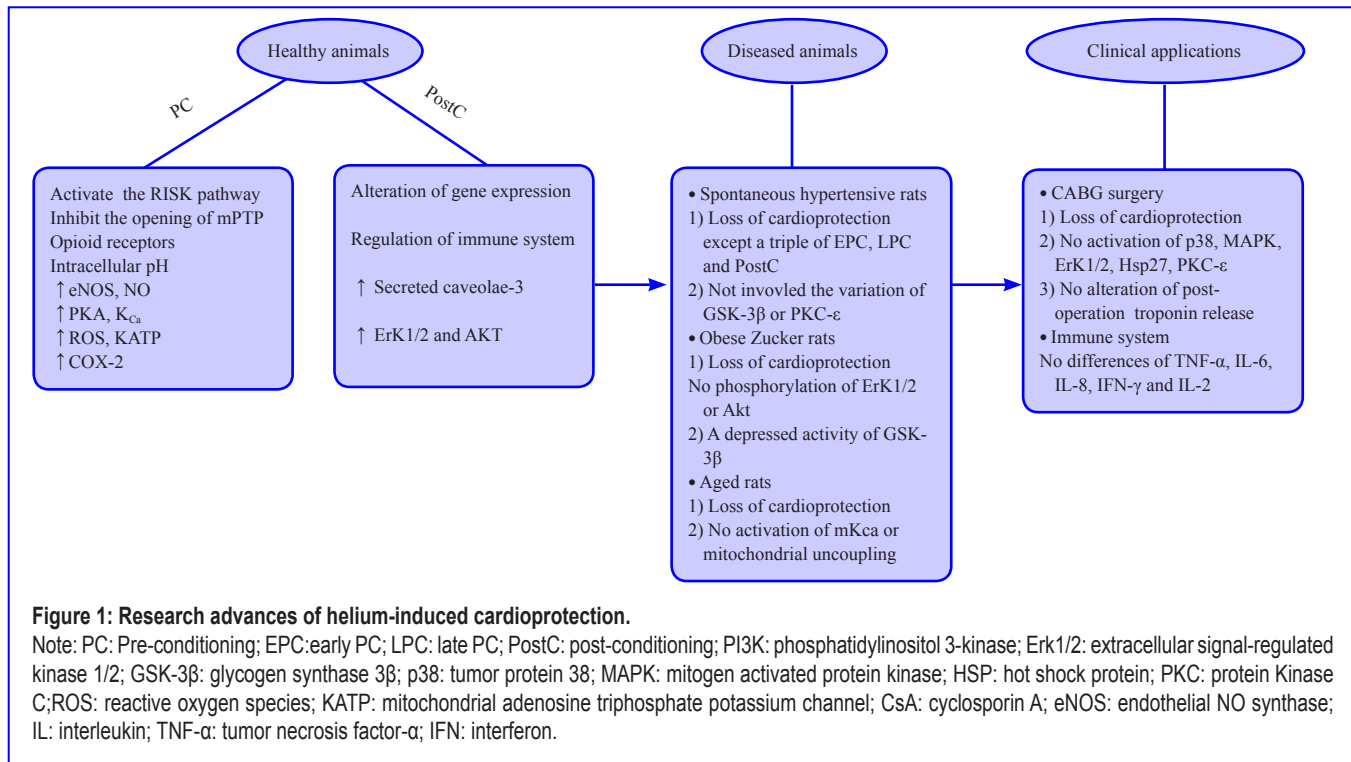
cytokines, TNF- α , IL-1 β , IL-6, and chemokine, interleukin-8 (IL-8), were measured after stimulation with LPS and LTA. The study suggests that prolonged inhalation of helium did not produce any influence on the ability of the innate and early adaptive immune system to respond to immune stimuli. There were no differences among TNF- α , IL-1 β , IL-6, IL-8, interferon- γ (IFN- γ) and interleukin-2 (IL-2) levels at different time points before and after helium inhalation in comparison to ordinary air inhalation.⁶⁹ Consequently, helium can hardly exert influence on the responsiveness of immune system.

We systematically summarize the research advances obtained from a variety of basic experiments related to the helium-induced cardioprotection so as to get more comprehensive and concise knowledge. The relevant evidences are described as **Figure 1**.

Although above evidences have been sufficient to fully explain helium played a significant role in myocardial ischemic injury, whether helium inhalation would exert a negative influence on the human body caused more or less anxiety. For instance, when the body is completely enclosed in helium, helium with high thermal conductivity may cause heat loss and thus reduce metabolism, so the energy consumption of the body is lower in a helium-filled environment.⁷⁰ What deserves our attention is that prolonging breathing by 75% of helium could induce hypothermia in rats.⁷¹ In addition, helium inhalation can cause distort voice because the density of helium is smaller than that of air and thus produce a higher resonance frequency to make the sound sharper.⁷² The above disadvantages may be a stumbling block for helium in the treatment of I/R injury, which makes people more hard to explore the most appropriate condition under which helium inhalation could minimize damage to the body.

CONCLUSION

From the above data we can conclude that multiple animal experiments suggest helium conditioning is associated with the inhibition of myocardial cell dysfunction and the reduction of myocardial I/R injury. The underlying mechanism of helium administration might involve the RISK pathway, the mPTP, the intracellular pH value, the caveolins and the immune system. The mechanisms by which these above-mentioned triggers, modulators and transducers generate favorable effects have not been completely elucidated, and abundant clinical researches are essential to prove their effectiveness and safety so as to translate the experimental data into clinical circumstances. As for how to alleviate the possible harm of helium inhalation on the organism, perhaps looking for the most suitable concentration, duration, timing and pause interval of helium inhalation is a good choice. In summary, helium is a promising alternative to protect the myocardium against I/R injury in the future investigations.



Author contributions

YPD and JYZ were responsible for writing the manuscript. DXF and GC were responsible for its revision. YK and ZX were responsible for its drafting and revision. All authors read and approved the final version of the paper for publication.

Conflicts of interest

The authors declare that they have no competing interests.

Open access statement

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Contributor agreement

A statement of “Publishing Agreement” has been signed by an authorized author on behalf of all authors prior to publication.

Plagiarism check

This paper has been checked twice with duplication-checking software iThenticate.

Peer review

A double-blind and stringent peer review process has been performed to ensure the integrity, quality and significance of this paper.

Open peer reviewer

Wen-wu Liu, Second Military Medical University, China.

REFERENCES

1. Weber NC, Smit KF, Hollmann MW, Preckel B. Targets involved in cardioprotection by the non-anesthetic noble gas helium. *Curr Drug Targets*. 2015;16:786-792.
2. Preckel B, Schlack W. Inert gases as the future inhalational anaesthetics? *Best Pract Res Clin Anaesthesiol*. 2005;19:365-379.
3. Dong X, Oganov AR, Goncharov AF, et al. A stable compound of helium and sodium at high pressure. *Nat Chem*. 2017;9:440-445.
4. Koblin DD, Fang Z, Eger EI 2nd, et al. Minimum alveolar concentrations of noble gases, nitrogen, and sulfur hexafluoride in rats: helium and neon as nonimmobilizers (nonanesthetics). *Anesth Analg*. 1998;87:419-424.
5. Oei GT, Weber NC, Hollmann MW, Preckel B. Cellular effects of helium in different organs. *Anesthesiology*. 2010;112:1503-1510.
6. Lique F, Kalugina Y, Chefdeville S, van de Meerakker SY, Costes M, Naulin C. Collisional excitation of O₂ by H₂: the validity of LTE models in interpreting O₂ observations. *Astron Astrophys*. 2014;567:A22.
7. Schmidt G, Keesom WH. New measurements of liquid helium temperatures: I. The boiling point of helium. *Physica*. 1937;4:963-970.
8. Uhlóa CA, Larner L. Helium-oxygen (Heliox) mixture in airway obstruction. *J Pediatr (Rio J)*. 2000;76:73-78.
9. Corcoran TE, Gamard S. Development of aerosol drug delivery with helium oxygen gas mixtures. *J Aerosol Med*. 2004;17:299-309.
10. Chen J, Cui C, Yang X, et al. MiR-126 affects brain-heart interaction after cerebral ischemic stroke. *Transl Stroke Res*. 2017. doi:10.1007/s12975-017-0520-z.



11. Bigam MT, Nowak JE, Wheeler DS. Therapeutic application of helium-oxygen and mechanical ventilation in a child with acute myelogenous leukemia and airway obstruction. *Pediatr Emerg Care.* 2009;25:469-472.
12. Hu HJ, Fan DF, Lv Y, et al. Effects of simulated heliox diving at high altitudes on blood cells, liver functions and renal functions. *Undersea Hyperb Med.* 2013;40:329-337.
13. Uchino H, Suzuki M, Okita A, et al. Organ protective effects of volatile anesthetics and perioperative outcomes. *Masui.* 2012;61:478-495.
14. Gannier M, Forel JM. Clinical review: use of helium-oxygen in critically ill patients. *Crit Care.* 2006;10:241.
15. Lucchinetti E, Wacker J, Maurer C, et al. Helium breathing provides modest antiinflammatory, but no endothelial protection against ischemia-reperfusion injury in humans in vivo. *Anesth Analg.* 2009;109:101-108.
16. Zhang R, Zhang L, Manaenko A, Ye Z, Liu W, Sun X. Helium preconditioning protects mouse liver against ischemia and reperfusion injury through the PI3K/Akt pathway. *J Hepatol.* 2014;61:1048-1055.
17. Rizvi M, Jawad N, Li Y, Vizcaychipi MP, Maze M, Ma D. Effect of noble gases on oxygen and glucose deprived injury in human tubular kidney cells. *Exp Biol Med (Maywood).* 2010;235:886-891.
18. Heinen A, Huhn R, Smeele KM, et al. Helium-induced preconditioning in young and old rat heart: impact of mitochondrial Ca(2+) -sensitive potassium channel activation. *Anesthesiology.* 2008;109:830-836.
19. Ahn JY, Tae HJ, Cho JH, et al. Activation of immediate-early response gene c-Fos protein in the rat paralimbic cortices after myocardial infarction. *Neural Regen Res.* 2015;10:1251-1257.
20. Kern KB, Hanna JM, Young HN, et al. Importance of both early reperfusion and therapeutic hypothermia in limiting myocardial infarct size post-cardiac arrest in a porcine model. *JACC Cardiovasc Interv.* 2016;9:2403-2412.
21. DeFily DV, Chilian WM. Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury. *Am J Physiol.* 1993;265:H700-706.
22. Hausenloy DJ, Yellon DM. The therapeutic potential of ischemic conditioning: an update. *Nat Rev Cardiol.* 2011;8:619-629.
23. Bolli R. The late phase of preconditioning. *Circ Res.* 2000;87:972-983.
24. Wang G, Li X, Wang H, et al. Later phase cardioprotection of ischemic post-conditioning against ischemia/reperfusion injury depends on iNOS and PI3K-Akt pathway. *Am J Transl Res.* 2015;7:2603-2611.
25. Yang F, Xi L. Postconditioning of ischemic heart by intermittent ventricular pacing at the beginning of reperfusion: novel mechanisms and potential utilities in interventional cardiology settings. *Am J Physiol Heart Circ Physiol.* 2016;310:H1-3.
26. Lupi Herrera E, Gaspar J, Gonzalez Pacheco H, et al. Reperfusion and postconditioning in acute ST segment elevation myocardial infarction. A new paradigm for the treatment of acute myocardial infarction. From bench to bedside? *Arch Cardiol Mex.* 2006;76 Suppl 4:S76-101.
27. Sandu N, Schaller B. Postconditioning: a new or old option after ischemic stroke? *Expert Rev Cardiovasc Ther.* 2010;8:479-482.
28. Pagel PS, Krolikowski JG, Shim YH, et al. Noble gases without anesthetic properties protect myocardium against infarction by activating prosurvival signaling kinases and inhibiting mitochondrial permeability transition in vivo. *Anesth Analg.* 2007;105:562-569.
29. Chipuk JE, Kuwana T, Bouchier-Hayes L, et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science.* 2004;303:1010-1014.
30. Juhaszova M, Zorov DB, Kim SH, et al. Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J Clin Invest.* 2004;113:1535-1549.
31. Hausenloy DJ, Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway. *Cardiovasc Res.* 2004;61:448-460.
32. Pagel PS, Krolikowski JG, Pratt PF Jr, et al. Inhibition of glycogen synthase kinase or the apoptotic protein p53 lowers the threshold of helium cardioprotection in vivo: the role of mitochondrial permeability transition. *Anesth Analg.* 2008;107:769-775.
33. Gross ER, Gross GJ. Ligand triggers of classical preconditioning and postconditioning. *Cardiovasc Res.* 2006;70:212-221.
34. Pagel PS, Krolikowski JG, Amour J, Warltier DC, Weihrauch D. Morphine reduces the threshold of helium preconditioning against myocardial infarction: the role of opioid receptors in rabbits. *J Cardiothorac Vasc Anesth.* 2009;23:619-624.
35. Kitakaze M, Weisfeldt ML, Marban E. Acidosis during early reperfusion prevents myocardial stunning in perfused ferret hearts. *J Clin Invest.* 1988;82:920-927.
36. Kitakaze M, Takashima S, Funaya H, et al. Temporary acidosis during reperfusion limits myocardial infarct size in dogs. *Am J Physiol.* 1997;272:H2071-2078.
37. Cohen MV, Yang XM, Downey JM. The pH hypothesis of postconditioning: staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. *Circulation.* 2007;115:1895-1903.
38. Pagel PS, Krolikowski JG. Transient metabolic alkalosis during early reperfusion abolishes helium preconditioning against myocardial infarction: restoration of cardioprotection by cyclosporin A in rabbits. *Anesth Analg.* 2009;108:1076-1082.
39. Tanaka K, Weihrauch D, Ludwig LM, Kersten JR, Pagel PS, Warltier DC. Mitochondrial adenosine triphosphate-regulated potassium channel opening acts as a trigger for isoflurane-induced preconditioning by generating reactive oxygen species. *Anesthesiology.* 2003;98:935-943.
40. Pagel PS, Krolikowski JG, Pratt PF Jr, et al. The mechanism of helium-induced preconditioning: a direct role for nitric oxide in rabbits. *Anesth Analg.* 2008;107:762-768.
41. Pagel PS, Krolikowski JG, Pratt PF Jr, et al. Reactive oxygen species and mitochondrial adenosine triphosphate-regulated potassium channels mediate helium-induced preconditioning against myocardial infarction in vivo. *J Cardiothorac Vasc Anesth.* 2008;22:554-559.
42. Huhn R, Weber NC, Preckel B, et al. Age-related loss of cardiac preconditioning: impact of protein kinase A. *Exp Gerontol.* 2012;47:116-121.



43. Fulton D, Gratton JP, McCabe TJ, et al. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature*. 1999;399:597-601.
44. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature*. 1999;399:601-605.
45. Feng J, Fischer G, Lucchinetti E, et al. Infarct-remodeled myocardium is receptive to protection by isoflurane postconditioning: role of protein kinase B/Akt signaling. *Anesthesiology*. 2006;104:1004-1014.
46. Ping P, Takano H, Zhang J, et al. Isoform-selective activation of protein kinase C by nitric oxide in the heart of conscious rabbits: a signaling mechanism for both nitric oxide-induced and ischemia-induced preconditioning. *Circ Res*. 1999;84:587-604.
47. Huhn R, Heinen A, Weber NC, et al. Helium-induced late preconditioning in the rat heart in vivo. *Br J Anaesth*. 2009;102:614-619.
48. Oei GT, Weber NC, Hollmann MW, Preckel B. Cellular effects of helium in different organs. *Anesthesiology*. 2010;112:1503-1510.
49. Oei GT, Huhn R, Heinen A, et al. Helium-induced cardioprotection of healthy and hypertensive rat myocardium in vivo. *Eur J Pharmacol*. 2012;684:125-131.
50. Huhn R, Heinen A, Weber NC, et al. Helium-induced early preconditioning and postconditioning are abolished in obese Zucker rats in vivo. *J Pharmacol Exp Ther*. 2009;329:600-607.
51. Oei GT, Heger M, van Golen RF, et al. Reduction of cardiac cell death after helium postconditioning in rats: transcriptional analysis of cell death and survival pathways. *Mol Med*. 2015;20:516-526.
52. Girm HR, Ahilathirunayagam S, Mavor AI, Homer-Vanniasinkam S. Reperfusion syndrome: cellular mechanisms of microvascular dysfunction and potential therapeutic strategies. *Vasc Endovascular Surg*. 2007;41:277-293.
53. Leichtweis S, Ji LL. Glutathione deficiency intensifies ischaemia-reperfusion induced cardiac dysfunction and oxidative stress. *Acta Physiol Scand*. 2001;172:1-10.
54. Timmers L, Pasterkamp G, de Hoog VC, Arslan F, Appelman Y, de Kleijn DP. The innate immune response in reperfused myocardium. *Cardiovasc Res*. 2012;94:276-283.
55. Glezer I, Simard AR, Rivest S. Neuroprotective role of the innate immune system by microglia. *Neuroscience*. 2007;147:867-883.
56. Ritter LS, Wilson DS, Williams SK, Copeland JG, McDonagh PF. Early in reperfusion following myocardial ischemia, leukocyte activation is necessary for venular adhesion but not capillary retention. *Microcirculation*. 1995;2:315-327.
57. Arslan F, de Kleijn DP, Pasterkamp G. Innate immune signaling in cardiac ischemia. *Nat Rev Cardiol*. 2011;8:292-300.
58. Oei GT, Aslami H, Kerindongo RP, et al. Prolonged helium postconditioning protocols during early reperfusion do not induce cardioprotection in the rat heart in vivo: role of inflammatory cytokines. *J Immunol Res*. 2015;2015:216798.
59. Patel HH, Murray F, Insel PA. Caveolae as organizers of pharmacologically relevant signal transduction molecules. *Annu Rev Pharmacol Toxicol*. 2008;48:359-391.
60. Hausenloy DJ, Yellon DM. Survival kinases in ischemic preconditioning and postconditioning. *Cardiovasc Res*. 2006;70:240-253.
61. Fridolfsson HN, Roth DM, Insel PA, Patel HH. Regulation of intracellular signaling and function by caveolin. *FASEB J*. 2014;28:3823-3831.
62. Patel HH, Head BP, Petersen HN, et al. Protection of adult rat cardiac myocytes from ischemic cell death: role of caveolar microdomains and delta-opioid receptors. *Am J Physiol Heart Circ Physiol*. 2006;291:H344-350.
63. Horikawa YT, Patel HH, Tsutsumi YM, et al. Caveolin-3 expression and caveolae are required for isoflurane-induced cardiac protection from hypoxia and ischemia/reperfusion injury. *J Mol Cell Cardiol*. 2008;44:123-130.
64. Flick M, Albrecht M, Oei GT, et al. Helium postconditioning regulates expression of caveolin-1 and -3 and induces RISK pathway activation after ischaemia/reperfusion in cardiac tissue of rats. *Eur J Pharmacol*. 2016;791:718-725.
65. Weber NC, van de Vondervoort D, Niesman IR, et al. Effects of noble gas conditioning on Caveolin expression in the rat heart in vivo. *FASEB J*. 2012;26:1114.17.
66. Weber NC, Schilling JM, Finley JC, et al. Helium inhalation induces caveolin secretion to blood. *FASEB J*. 2013;27:1089.3.
67. Smit KF, Brevoord D, De Hert S, et al. Effect of helium pre- or postconditioning on signal transduction kinases in patients undergoing coronary artery bypass graft surgery. *J Transl Med*. 2016;14:294.
68. Smit KF, Brevoord D, de Hert SG, Hollmann MW, Weber NC. Helium induced pre and postconditioning in patients subjected to coronary artery bypass graft (CABG) surgery: 4AP15. *Eur J Anaesthesiol*. 2012;29:53.
69. Oei GT, Smit KF, vd Vondervoort D, et al. Effects of helium and air inhalation on the innate and early adaptive immune system in healthy volunteers ex vivo. *J Transl Med*. 2012;10:201.
70. Singer D. Why 37 degrees C? Evolutionary fundamentals of thermoregulation. *Anaesthesist*. 2007;56:899-902, 904-896.
71. David HN, Haelewyn B, Chazalviel L, et al. Post-ischemic helium provides neuroprotection in rats subjected to middle cerebral artery occlusion-induced ischemia by producing hypothermia. *J Cereb Blood Flow Metab*. 2009;29:1159-1165.
72. Montgomery C. Why does inhaling helium make one's voice sound strange? *Sci Am*. 2004;291:122.