Helium Breathing Provides Modest Antiinflammatory, but No Endothelial Protection Against Ischemia-Reperfusion Injury in Humans *In Vivo*

Eliana Lucchinetti, PhD*

Johannes Wacker, MD†

Christian Maurer, MD†

Marius Keel, MD‡

Luc Härter, PhD‡

Kathrin Zaugg, MD, PhD§

Michael Zaugg, MD‖

BACKGROUND: The noble gas helium is devoid of anesthetic effects, and it elicits cardiac preconditioning. We hypothesized that inhalation of helium provides protection against postocclusive endothelial dysfunction after ischemia-reperfusion of the forearm in humans.

METHODS: Eight healthy male subjects were enrolled in this study with a crossover design. Each volunteer was randomly exposed to 15 min of forearm ischemia in the presence or absence of helium inhalation. Helium was inhaled at an end-tidal concentration of 50 vol% from 15 min before ischemia until 5 min after the onset of reperfusion ("helium conditioning"). Hyperemic reaction, a marker of nitric oxide bioavailability and endothelial function, was determined at 15 and 30 min of reperfusion on the forearm using venous occlusion plethysmography. Expression of the proinflammatory markers CD11b, ICAM-1, PSGL-1, and L-selectin (CD62L) on leukocytes and P-selectin (CD62P), PSGL-1, and CD42b on platelets were measured by flow cytometry during reperfusion.

RESULTS: Ischemia-reperfusion consistently reduced the postocclusive endothelium-dependent hyperemic reaction at 15 and 30 min of reperfusion. Periischemic inhalation of helium at 50 vol% did not improve postocclusive hyperemic reaction. Helium decreased expression of the proinflammatory marker CD11b and ICAM-1 on leukocytes and attenuated the expression of the procoagulant markers CD42b and PSGL-1 on platelets.

CONCLUSIONS: Although inhalation of helium diminished the postischemic inflammatory reaction, our data indicate that human endothelium, which is a component of all vital organs, is not amenable to protection by helium at 50 vol% *in vivo*. This is in contrast to sevoflurane, which protects human endothelium at low subanesthetic concentrations.

(Anesth Analg 2009;109:101–8)

Endothelium-mediated vasodilator response is markedly diminished by mental and physical stress, pain, diabetes, hypercholesterolemia, and hypertension.[1–3] The same conditions are also associated with an increased incidence of perioperative cardiovascular complications.[4,5] This is not surprising as endothelial dysfunction during ischemia-reperfusion exacerbates vasospasm and thus is a critical determinant of the extent of ischemic organ injury.[6,7] Because the endothelium is a key component of all vital organs, it can be speculated that interventions aiming at improving endothelial function potentially protect the whole body.

Using a human forearm model of simulated ischemia-reperfusion injury, we recently demonstrated that the halogenated ether sevoflurane administered at low subanesthetic but sedative doses (≤1 vol%) abolished postocclusive endothelial dysfunction and activation of inflammatory white blood cells.[8] Small-dose sevoflurane inhalation was further capable of inhibiting agonist-induced formation of thrombogenic granulocyte-platelet aggregates[9] and of decreasing the expression of the proinflammatory L-selectin 24 h after sevoflurane inhalation in volunteers,[10] which is consistent with the occurrence of a "second window of protection" in humans. An increasing body of evidence now suggests that the noble gas helium, which

From the *Department of Anesthesiology and Pain Medicine, University of Alberta, Edmonton, Canada; †Institute of Anesthesiology, ‡Department of Trauma Surgery, §Department of Radiation Oncology, University Hospital Zurich, Zurich, Switzerland; and ‖Department of Anesthesiology and Pain Medicine, University of Alberta and Perioperative Translational Medicine, Mazankowski Alberta Heart Institute, Edmonton, Canada.

Accepted for publication January 7, 2009.

Supported by the Grant #3200B0-103980/1 of the Swiss National Science Foundation, Berne, Switzerland, the Swiss Society of Anesthesiology and Reanimation, Berne, Switzerland, the 5th Frontiers in Anesthesia Research Award from the International Anesthesia Research Society, Cleveland, OH, and a grant from the Mazankowski Alberta Heart Institute, Edmonton, Canada.

EL, JW, KZ, and MZ contributed similarly to this work.

Please see supplementary material available at www.anesthesia-analgesia.org.

Reprints will not be available from the author.

Address correspondence to Michael Zaugg, MD, DEAA, FRCPC, Department of Anesthesiology and Pain Medicine, University of Alberta, CSB Room 8-120, Edmonton AB T6G 2G3. Address e-mail to michael.zaugg@ualberta.ca.

DOI: 10.1213/ane.0b013e3181a27e4b

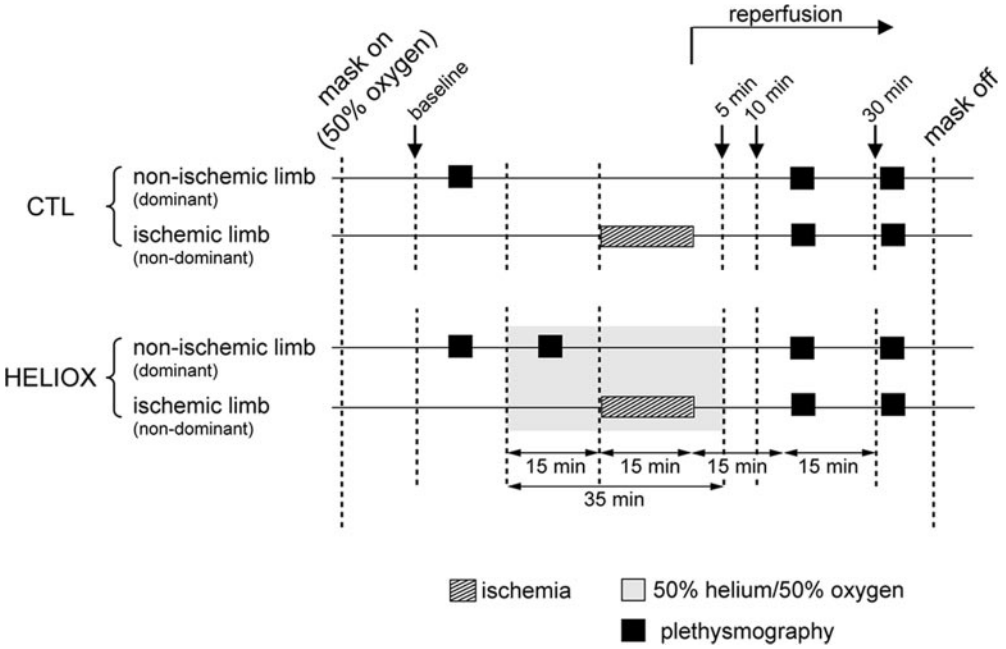

Figure 1. Study protocols. The forearm was rendered hypoxic for 15 min. Hyperemic blood flow response was measured using venous occlusion plethysmography after 15 and 30 min of reperfusion to assess endothelial function. Blood samples for flow cytometry were collected after 5, 10, and 30 min of reperfusion. CTL = control protocol.

is devoid of any sedative effects, may provide similar and efficient protection in the heart by activating signaling pathways previously described for halogenated ethers.[11–13] Experimental data imply that helium concentrations well below 50 vol% are cardioprotective. However, it is unclear whether helium specifically protects the endothelium in humans similar to halogenated ethers[8] or, in other words, whether endothelial protection is a component of helium-induced organ protection. Therefore, using our previously established model,[8] we tested in volunteers whether helium could prevent endothelial dysfunction *in vivo* and, if so, whether this protection would be related to a reduced expression of proinflammatory markers on leukocytes and platelets. Specifically, we hypothesized that periischemic helium inhalation, i.e., "helium conditioning," would improve postocclusive endothelial dysfunction by inhibiting the inflammatory response on white blood cells.

METHODS

Study Subjects

This study was performed in accordance with the Declaration of Helsinki (2000) and was approved by the local ethics committee. Eight healthy male volunteers (35 ± 7 (25–45) yr) with a Body Mass Index of 21 ± 2 kg/m^2 and normal hematological variables (hemoglobin 14.5 ± 0.5 g/dL, leukocytes 5.54 ± 0.82 × 10^3 per μL, platelets 245 ± 34 × 10^3 per μL) gave informed signed consent. All subjects were nonsmokers and refrained from caffeine and dark chocolate 24 h before study participation.

Study Protocol

Ischemia-reperfusion of the forearm was used as a model of endothelial dysfunction.[7,8,14] Figure 1 depicts the time course of the experiments. An IV cubital line was placed on the nondominant arm, and 100 mL Ringer's solution was administered. Studies were performed in a temperature-controlled and quiet room (24°C) during the early morning hours. The nondominant forearm was rendered ischemic for 15 min by inflating a 12-cm-wide blood pressure cuff placed around the upper arm to 180 mm Hg to induce endothelial dysfunction (index ischemia). In all protocols, the spontaneously breathing volunteers used a tightly fitting cushioned face mask connected to the common gas outlet of an anesthesia machine (Siemens Servo 900D ventilator; Siemens Life Support Systems, Sona, Sweden). To overcome the internal resistance of the ventilator and connected tubing and to obtain the most convenient conditions for each volunteer, a pressure support of 2–4 mbar was used and the inspiration trigger was set to 0–3 mbar. At the beginning and at the end of the protocols, all subjects inhaled nitrogen in 50 vol% oxygen (Fig. 1). Helium (Messer Schweiz AG, Lenzburg, Switzerland) or nitrogen in 50 vol% oxygen was inhaled from 15 min before forearm ischemia until 5 min after reperfusion. Blood samples were taken from the cubital vein of the nondominant arm before test ischemia (baseline) and after 5, 10, and 30 min of reperfusion. Monitoring consisted of intermittent noninvasive arterial blood pressure measurements, 5-lead electrocardiogram, end-tidal CO_2,

O_2, and N_2 concentrations (Infinity Delta XL, Draeger Medical Systems, Danvers, MA).

Assessment of Resistance Vessel Endothelial Function Using Hyperemic Blood Flow Response in the Forearm

We have previously described this procedure in detail.[8] Mercury-in-silastic strain-gauge venous occlusion plethysmography (Vasoquant 4000; ELCAT GmbH, Wolfratshausen, Germany), which is considered to be the "gold standard" for the early detection of endothelial dysfunction, was used to measure the hyperemic blood flow response in the forearm.[15–18] Briefly, volunteers were placed in a supine position with both arms extended and elevated. Venous congestion was achieved by inflating a cuff around the upper arm to 40 mm Hg. The recorded period of blood flow consisted of four cycles of venous occlusion followed by deflation (each 5 s) while the hands were excluded from the circulation. Reactive hyperemia was induced by 4 min of blood flow arrest using a blood pressure cuff inflated 20 mm Hg above the systolic blood pressure. Early hyperemic reaction (EHR, peak flow at the onset of reperfusion representing the first measurement after deflation of the cuff) and late hyperemic reaction (LHR, maintenance of hyperemia representing the mean of the three subsequent measurements) were recorded. Reactive hyperemia was determined at baseline before index ischemia on the dominant nonexperimental forearm to avoid ischemic preconditioning before the experiment, 15 and 30 min after test ischemia on both forearms. In the helium protocol, reactive hyperemia was also determined 5 min after initiation of helium inhalation to test whether helium itself without prior index ischemia would change the hyperemic blood flow response.

Determination of Proinflammatory (CD11b, ICAM-1, CD62L, and PSGL-1) and Procoagulant (CD42b and CD62P) Markers Using Flow Cytometry

Heparinized blood samples were immediately processed for flow cytometry. The expression of the proinflammatory molecules was determined at baseline, 5, 10, and 30 min during reperfusion in all eight volunteers.[8,9] Three microliters of the primary fluorochrome-labeled antibody was added to 50 μL of blood in endotoxin-free tubes and incubated in the dark for 10 min at room temperature. Lysis buffer (450 μL) (Becton Dickinson, Basel Switzerland) was added and incubated for an additional 20 min at room temperature. The lysates were fixed for 30 min in 0.5 mL 0.2% paraformaldehyde solution at room temperature. The samples were centrifuged, and the cell pellets were suspended in 0.5 mL of TLR buffer and stored at 4°C in the dark. The FACSCalibur (Becton Dickinson, Basel Switzerland) flow cytometer was used to measure R-phycoerythrin (PE)-fluorescence at 580 nm and FITC-fluorescence at 515 nm. White blood cells and platelets were distinguished from each other by typical physical characteristics, resulting in well-delineated cellular subpopulations that are easily identified on forward and side-scatter plots. Monoclonal antibodies for polymorphnuclear granulocytes (CD15, PE-labeled, clone 80H5, Immunotech, Marseille France), monocytes (CD14, FITC-labeled, clone 61D3, eBioscience, Wembley, UK), and platelets (CD62P, PE-labeled, clone AK-4, Santa Cruz Biotechnology, CA) further served for identification of cellular subgroups. The following additional surface markers were used: CD62L (FITC-labeled, clone AN51, DAKO, Glostrup Denmark), CD11b (PE-labeled, clone 2LPM19C, DAKO, Glostrup Denmark), ICAM-1 (FITC-labeled, clone sc-107, Santa Cruz Biotechnology, CA), PSGL-1 (FITC-labeled, clone sc-32302, Santa Cruz Biotechnology, CA), and CD42b (GPIIb, clone AN51, FITC-labeled, DAKO, Glostrup Denmark). Results were compared with isotype-matched antibodies staining as controls (PE-labeled IgG, eBioscience, Wembley, UK and FITC-labeled IgG, Becton Dickinson, Basel, Switzerland). A minimum of 20,000 events was counted on each sample. Data are shown as fold-change of mean fluorescence intensities (MFIs) from the respective baseline values.

Statistical Analysis

Forearm blood flow was measured in milliliters per 100 g tissue per minute and expressed as percent change compared with baseline flow measurements. The sample size was calculated based on published data of endothelial protection by sevoflurane in humans, as measured by venous occlusion plethysmography.[8] With an expected difference of 50% between group means (percent change from baseline), i.e., between the control protocol without helium inhalation and the protocol with helium inhalation (Heliox), and a standard deviation of 30% in each group, an $\alpha = 0.05$, and a $\beta = 0.80$, a sample size of eight volunteers (control and treatment arm) was necessary in this study with a crossover design. For each participating subject, activation of leukocytes, and platelets on the ischemic limb was expressed as the ratio of MFIs between time-matched control and helium blood samples of the investigated surface markers, as follows:

$$\text{ratio}(\text{subject } S_i;\ \text{timepoint } t_j) = \frac{MFI_{S_i\ HELIOX}(t_j)}{MFI_{S_i\ CTL}(t_j)},$$

where i = 1, 2, . . . 8 and j = baseline, 5 min, 10 min, 30 min

Data were subsequently normalized to baseline.[8] Paired *t*-test and repeated-measures analysis of variance were used for comparison. $P < 0.05$ was considered significant. Data are given as mean ± SD after testing for normality. Analyses were performed using SigmaStat Version 2 (SPSS Science, Chicago, IL).

Table 1. Recorded Variables During Inhalation of Gas Mixtures

Subjects	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
Before Helium								
SBP (mm Hg)	133	110	114	112	126	128	116	120
HR (bpm)	73	60	56	60	56	58	70	73
Spo_2 (%)	100	99	100	99	100	100	100	100
During Helium								
SBP (mm Hg)	130	111	110	105	130	132	117	104
HR (bpm)	66	65	56	52	55	58	60	70
Spo_2 (%)	100	100	99	100	100	100	99	100
ETo_2 (vol%)	42	44	45	47	43	45	46	45
$ETco_2$ (vol%)	5	4.8	4.4	4.5	4.2	5.2	3.8	3.7

SBP = systolic blood pressure at baseline and 10 min after initiation of helium (50 vol%) inhalation; HR = mean heart rate; ETo_2= mean end-tidal O_2 concentration; $ETco_2$ = mean end-tidal CO_2; Spo_2 = mean oxygen saturation (finger tip); bpm = beats per minute; V1–8 = individual volunteers.

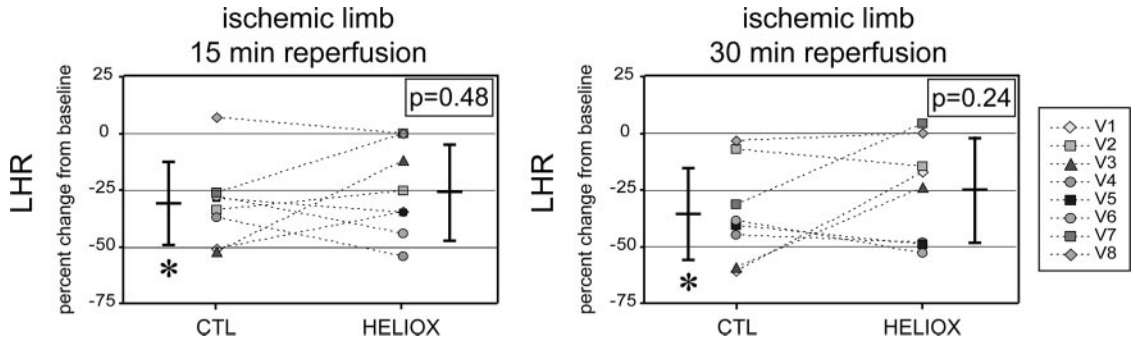

Figure 2. Late hyperemic blood flow response (LHR) in the ischemic arm. The percent change in blood flow is shown for each subject at 15 and 30 min after test ischemia. Mean values and SDs are depicted next to the individual measurements. There was a significant reduction in LHR after ischemia-reperfusion injury versus baseline (*$P < 0.05$). However, helium breathing did not improve postocclusive hyperemic reaction. CTL = control protocol; V1–8 = individual volunteers.

RESULTS

All study subjects tolerated the procedures well without complications. The 35 min period of helium inhalation at 50 vol% had no effect on arterial blood pressure, heart rate, and end-tidal CO_2 concentrations (Table 1). There was no sedation in any of the participants, nor was there any analgesic effect on the discomfort associated with the 15 min of ischemia-reperfusion of the forearm when helium was inhaled compared with nitrogen in the control protocol. Despite equal ventilation settings in the control and helium protocols for each of the eight participants, seven of them reported a subjective reduction in the resistive work of breathing during helium inhalation.

Ischemia-Reperfusion in the Forearm Markedly Reduces Endothelium-Dependent Hyperemic Blood Flow Response in Humans

Fifteen minutes of ischemia followed by reperfusion was used to induce endothelial dysfunction. Hyperemic blood flow response was measured by venous occlusion plethysmography after 15 and 30 min of reperfusion on both the ischemic and the nonischemic side. Although peak flow at reopening of the forearm vessels (EHR) was unaffected by test ischemia when compared with baseline (at 15 min of reperfusion: −6% ± 23%, at 30 min of reperfusion: −12% ± 15%), maintenance of hyperemic reaction (LHR) was markedly ($P < 0.05$) reduced versus baseline (at 15 min of reperfusion: −27% ± 15%, at 30 min of reperfusion: −29% ± 12%) (Fig. 2). Hyperemic blood flow response was unaffected by index ischemia on the nonischemic control arm, i.e., there was no induction of "remote" effects on the neighboring endothelium after ischemia-reperfusion injury (data not shown). Original tissue flow measurements are available in Supplementary Table S1, available at www.anesthesia-analgesia.org.

Periischemic Breathing of Helium does not Restore Postocclusive Endothelial Dysfunction of the Forearm in Humans

Each volunteer underwent the same procedure with and without helium inhalation in a random fashion. Helium was inhaled from 15 min before ischemia until 5 min after reperfusion, mimicking a combination of pre- and postconditioning ("conditioning"). There was no postocclusive improvement of LHR (Fig. 2). Interestingly, helium itself did not affect EHR versus baseline (3% ± 6%) or LHR versus baseline (−1% ± 11%), nor did it affect EHR on the nonischemic limb versus control after index ischemia (at 15 min of reperfusion with helium 1% ± 8% vs 5% ± 11% in control, at 30 min of reperfusion with helium −4% ± 12% vs 5% ± 3% in control) or LHR on the nonischemic arm after index ischemia (at 15 min of reperfusion with helium 4% ± 15% vs 2% ± 17% in

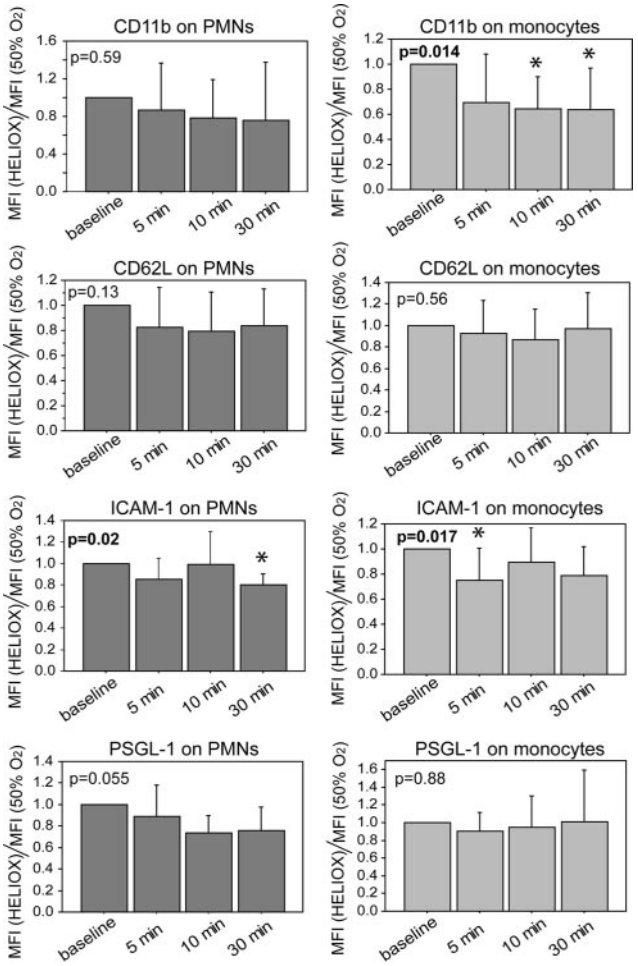

Figure 3. Proinflammatory markers on leukocytes. CD11b, CD62L (L-selectin), ICAM-1, and PSGL-1 expressions were used to assess leukocyte activation after ischemia-reperfusion injury in the ischemic arm. There was only a modest decrease in CD11b and ICAM-1 expression with helium inhalation. Data are mean ± SD. CTL = control protocol; PMNs = polymorphnuclear granulocytes; MFI = mean fluorescence intensity; HELIOX = 50 vol% helium/50 vol% oxygen. *$P < 0.05$ versus baseline.

control, at 30 min of reperfusion with helium 0% ± 23% vs −1% ± 21% in control). These data further exemplify the inability of helium to directly affect endothelial function in humans.

Helium Breathing only Modestly Reduces the Proinflammatory and Procoagulant Cell Surface Markers After Ischemia-Reperfusion Injury

To test whether helium affects the expression of the proinflammatory markers on leukocytes and the procoagulant markers on platelets, blood was collected during reperfusion from the injured side and compared with baseline conditions. Baseline flow cytometry data are available in Supplementary Table S2, available at www.anesthesia-analgesia.org. The markers were expressed as the ratio of MFI between time-matched control and helium blood samples. Helium reduced the ratio of CD11b expression on monocytes and ICAM-1 on granulocytes and monocytes during reperfusion (Fig. 3). Although no changes in CD62P (P-selectin) expression were observed, reductions in PSGL-1 and CD42b expressions on platelets were detected during reperfusion (Fig. 4). Because this study used the identical experimental protocols to assess endothelial function, as previously reported for sevoflurane by our group,[8] we directly compared the endothelial protection by helium (50 vol%) with sevoflurane (≤1 vol%). The comparison shows that helium can only provide modest antiinflammatory actions against ischemia-reperfusion injury. However, it is incapable of improving postocclusive endothelial dysfunction in humans (Fig. 5).

DISCUSSION

The present study shows the following salient findings. First, in contrast to our hypothesis, helium inhalation in humans elicited no postocclusive improvement of endothelial dysfunction after ischemia-reperfusion injury on the forearm. Moreover, and unlike previous observations with sevoflurane,[8] helium did not affect vasomotion on the nonischemic limb, indicating no remote systemic "preconditioning-like" effects on the vasculature. Second, although helium modestly attenuated the postischemic expression of inflammatory cell surface markers on leukocytes and platelets, it was unable to restore endothelial function at the used concentration (50 vol%). Nonetheless, the observed antiinflammatory and anticoagulant actions elicited by helium may be, at least in part, responsible for its previously reported organ protective effects.[12,13,19]

Venous occlusion plethysmography is a reliable noninvasive tool to investigate vascular function and is regarded to be the gold standard for evaluating endothelial function.[16,17] In the present study, we determined reactive hyperemia, which is mediated by the endothelial release of nitric oxide,[20] using venous occlusion plethysmography after prolonged test ischemia of the forearm. With the aid of this well-established model,[1,7,8] we previously showed that hyperemic blood flow remains unchanged after nitroglycerin application consistent with an endothelium-dependent, but smooth muscle-independent, vasomotion effect.[8] These studies further demonstrated that late, as opposed to early hyperemic blood flow response, more reliably reflects endothelial function and vascular integrity. The findings of the present study show that helium, as opposed to sevoflurane, is unable to prevent postocclusive endothelial dysfunction.

Helium is a biologically inert noble gas without anesthetic properties.[21,22] Its application as an oxygen carrier in the clinical setting is largely based on its low density (−250% if nitrogen in air is replaced by helium) and rapid diffusion due to its low atomic weight, as compared with nitrogen, which markedly reduces the resistive work of breathing.[23] Improved CO_2 elimination and reduced oxygen requirements combined with antiinflammatory actions lead to an

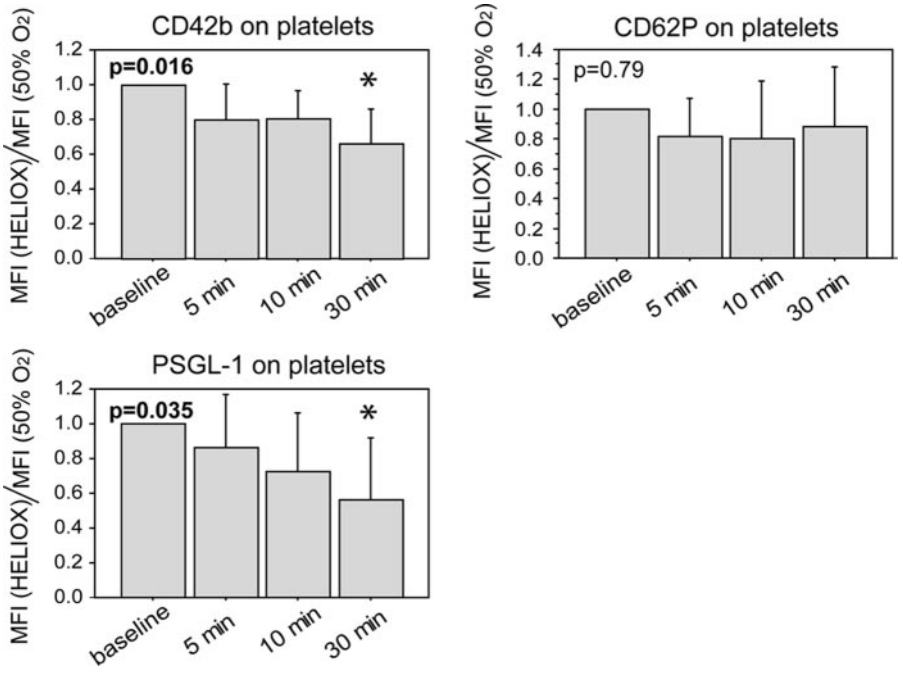

Figure 4. Procoagulant markers on platelets. CD42b, CD62P (P-selectin), and PSGL-1 expressions were used to assess platelet activation after ischemia-reperfusion injury in the ischemic arm. There was a significant decrease in CD42b and PSGL-1 expression with helium inhalation. Data are mean ± SD. CTL = control protocol; MFI = mean fluorescence intensity; HELIOX = 50 vol% helium/50 vol% oxygen. *$P < 0.05$ versus baseline.

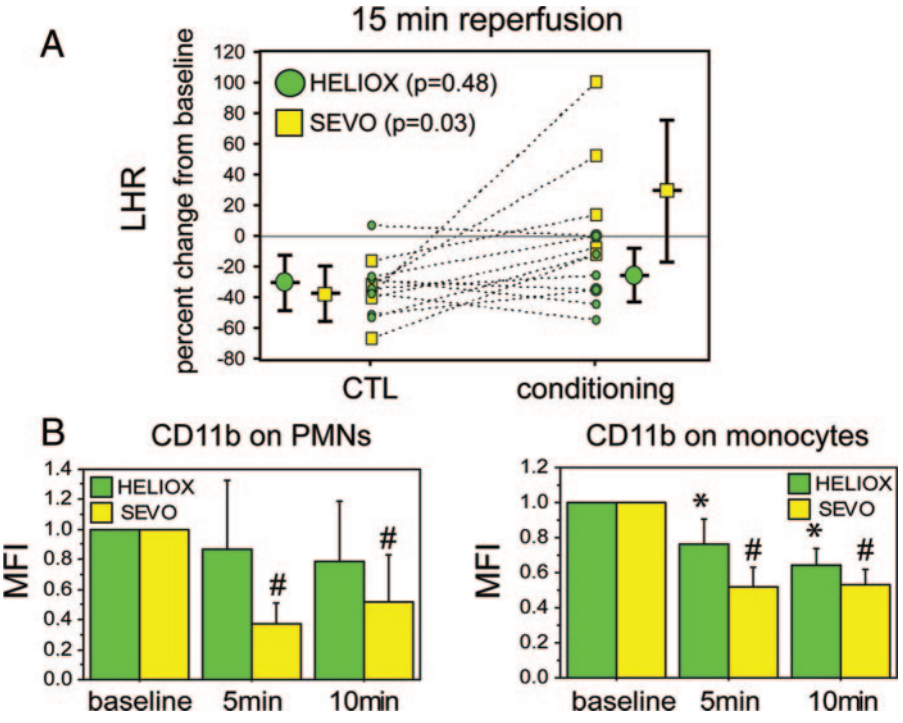

Figure 5. Effects of helium and sevoflurane inhalation on endothelial protection. Using the identical model of ischemia-reperfusion injury on the forearm of volunteers in a crossover study, sevoflurane (<1 vol%) but not helium (50 vol%) prevented postocclusive endothelial dysfunction. Panel A: Late hyperemic reaction (LHR). Mean values and standard deviations are depicted next to the individual measurements. $n = 5$ for sevoflurane (SEVO), $n = 8$ for HELIOX (50 vol% helium/50 vol% oxygen). Sevoflurane data are reproduced from Ref. 8 Figure 2B. Panel B: CD11b expression on polymorphnuclear granulocytes (PMNs) and monocytes. *$P < 0.05$ versus baseline. #$P < 0.05$ versus time-matched HELIOX sample. Sevoflurane data are reproduced from Lucchinetti et al.[8] with permission from Wolters Kluwer Health, Figure 5A and 5B. Data are mean ± SD.

increased efficiency of ventilation and suggest a potential lung-protective strategy.[19] Recently, the nonsedative helium also emerged as an attractive preconditioning-mimicking agent in other vital organs. Using helium inhalation in an *in vivo* rat model of focal brain ischemia, Pan et al.[12] reported a reduction in infarct size, as assessed by triphenyltetrazolium staining, from 36% to 4% after a middle cerebral artery occlusion for 2 h and a reperfusion period of 1 h. Similarly, using an *in vivo* rabbit model of coronary artery ligation, Pagel et al.[13] reported an infarct size reduction by 50% (from 45% to 23%) if three cycles of helium breathing were administered before a lethal ischemia of 3 h. Blocker experiments from these studies suggest that helium activates prosurvival kinases and mitochondrial K_{ATP} channels, ultimately inhibiting mitochondrial permeability transition pore opening,[24] as previously reported for halogenated ethers.[25,26] In other experimental studies comparing helium versus carbon dioxide pneumoperitoneum, helium ameliorated pneumoperitoneum-associated renal dysfunction[27] and reduced tumor recurrence and spreading.[28,29] Interestingly, in our study helium breathing predominantly decreased ICAM-1 expression on leukocytes, a cellular surface marker that plays a pivotal

role in leukocyte-endothelium interaction.[30] Also, the adhesion promoting PSGL-1, mainly expressed on leukocytes but also on activated platelets,[31] and to a lesser degree the platelet receptor for the von Willebrand factor (CD42b), were diminished by periischemic helium inhalation. In contrast to these promising reports and despite the fact that vascular endothelium and leukocyte- and platelet-endothelial interactions are critically involved in many steps of tissue damage originating from ischemia-reperfusion injury, our human *in vivo* study could only show modest helium-induced antiinflammatory effects, which were not capable of preventing ischemia-induced endothelial dysfunction. With respect to our previous study on endothelial protection by sevoflurane,[8] it could be speculated that either sevoflurane-induced endothelial preconditioning and antiinflammatory actions are mediated by separate pathways (of which helium also uses the latter), or the mild antiinflammatory actions of helium are mediated by pathways distinct from those of sevoflurane and apparently are unrelated to endothelial protection by preconditioning. Nevertheless, these beneficial actions of helium could still contribute to the previously reported organ protection[12,13,19,27] elicited by helium and potentially provide synergism in protection if helium was combined with halogenated ethers. In fact, helium as a carrier gas for halogenated ethers[32,33] and oxygen may bring along several substantial advantages. First, the endothelial protective but yet sedative dose of approximately 1/2 minimum alveolar concentration[8] required for sevoflurane-induced (endothelial) protection could be markedly reduced, although still maintaining the same degree of protection. Second, because of the optimized respiratory mechanics and the specific gas properties of helium, airway patency could be guaranteed more safely. Third, denitrogenation by itself might protect mitochondria from ischemia-reperfusion injury, because swelling of these organelles from nitrogen is thought to be a possible cause of reperfusion damage.[34] Hence, these obvious advantages make the implementation of helium into a protective inhalation treatment strategy highly attractive in nonsedated at-risk patients undergoing coronary or vascular interventions. However, helium bears a certain risk of hypothermia (fivefold higher conductivity than air), diffusion hypoxia (33-fold lower solubility than nitrous oxide), tension pneumothorax and bubble formation, as observed in decompression sickness.

Specific Remarks and Study Limitations:

To overcome interindividual variability, we have chosen a crossover study design, and confounding variables were carefully excluded. As observed in our own data, some long-term (days to weeks) reproducibility studies evaluating venous occlusion plethysmography over time showed relatively high coefficients of variation (20%–40%), mainly because of the different placement of the strain-gauge and venous occlusion cuff on the forearm.[17] However, short-term (hours) reproducibility of venous occlusion plethysmography, particularly without manipulating the strain-gauge and blood pressure cuff, is excellent (5%–10%).[17] Also, marked changes in absolute values of basal forearm blood flow in an individual may occur as a result of changes in sympathetic tone.[15] Although these changes may affect absolute responses to interventions, they do not alter the percent response to interventions calculated from the ratio of blood flow. Thus, the same intervention in a single subject recorded on different days gives similar results. Conversely, observed differences in the ratio of blood flow of the same individual in response to a specific intervention during two protocols recorded on different days are likely to be true treatment effects. In our study, the postischemic tissue perfusion measurements used for comparison with baseline perfusion were recorded within 1 h, and the percent changes were subsequently compared between the control and the helium protocol. Hence, it is unlikely that long-term variations in tissue perfusion may have biased our results. There was a small improvement in LHR on an average at 15 min of reperfusion after helium inhalation, and four of eight volunteers showed an increase in LHR after helium inhalation. If these small changes were consistently detectable and clinically relevant, our study would be under-powered. However, despite helium inhalation, tissue perfusion remained below baseline conditions after ischemia-reperfusion injury in three of four volunteers, who showed small improvements, indicating the failure of this therapy to restore endothelial function and to normalize postischemic tissue perfusion. In the present study, we used helium breathing at 50 vol%. Therefore, we cannot completely exclude that helium inhalation at 70 vol% or higher concentrations might provide endothelial protection. However, gas mixtures with low oxygen content are unlikely to be acceptable by clinicians, particularly in at-risk patients suffering from ischemia-reperfusion injury. Because our ischemia forearm model does not induce necrosis of endothelial cells, but rather a state of "endothelial stunning," which resolves spontaneously within 60 min,[15,18] it is unlikely that more substantial endothelial injury, which usually occurs in clinical situations, might be protected by helium.

In conclusion, helium inhalation at 50 vol% is unable to specifically provide protection to the endothelium in humans. However, future studies inpatients should evaluate the potential synergism in vital organ protection between helium and the highly protective halogenated ethers.

ACKNOWLEDGMENTS

The authors thank the PACU nurses, colleagues, and volunteers, who participated in this study.

REFERENCES

1. Ghiadoni L, Donald AE, Cropley M, Mullen MJ, Oakley G, Taylor M, O'Connor G, Betteridge J, Klein N, Steptoe A, Deanfield JE. Mental stress induces transient endothelial dysfunction in humans. Circulation 2000;102:2473–8
2. Jambrik Z, Santarcangelo EL, Rudisch T, Varga A, Forster T, Carli G. Modulation of pain-induced endothelial dysfunction by hypnotisability. Pain 2005;116:181–6
3. Sanada H, Higashi Y, Goto C, Chayama K, Yoshizumi M, Sueda T. Vascular function in patients with lower extremity peripheral arterial disease: a comparison of functions in upper and lower extremities. Atherosclerosis 2005;178:179–85
4. Zaugg M, Schaub MC, Foex P. Myocardial injury and its prevention in the perioperative setting. Br J Anaesth 2004; 93:21–33
5. Zaugg M, Schulz C, Wacker J, Schaub MC. Sympathomodulatory therapies in perioperative medicine. Br J Anaesth 2004;93:53–62
6. Hearse DJ. Myocardial protection during ischemia and reperfusion. Mol Cell Biochem 1998;186:177–84
7. Kharbanda RK, Peters M, Walton B, Kattenhorn M, Mullen M, Klein N, Vallance P, Deanfield J, MacAllister R. Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans in vivo. Circulation 2001;103:1624–30
8. Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Harter L, Keel M, Meier T, Zaugg M. Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. Anesthesiology 2007;106:262–8
9. Wacker J, Lucchinetti E, Jamnicki M, Aguirre J, Härter L, Keel M, Zaugg M. Delayed inhibition of agonist-induced granulocyte-platelet aggregation after low-dose sevoflurane inhalation in humans. Anesth Analg 2008;106:1749–58
10. Lucchinetti E, Aguirre J, Feng J, Zhu M, Suter M, Spahn DR, Harter L, Zaugg M. Molecular evidence of late preconditioning after sevoflurane inhalation in healthy volunteers. Anesth Analg 2007;105:629–40
11. Zaugg M, Lucchinetti E, Uecker M, Pasch T, Schaub MC. Anaesthetics and cardiac preconditioning. Part I. Signalling and cytoprotective mechanisms. Br J Anaesth 2003;91:551–65
12. Pan Y, Zhang H, VanDeripe DR, Cruz-Flores S, Panneton WM. Heliox and oxygen reduce infarct volume in a rat model of focal ischemia. Exp Neurol 2007;205:587–90
13. Pagel PS, Krolikowski JG, Shim YH, Venkatapuram S, Kersten JR, Weihrauch D, Warltier DC, Pratt PF Jr. Noble gases without anesthetic properties protect myocardium against infarction by activating prosurvival signaling kinases and inhibiting mitochondrial permeability transition in vivo. Anesth Analg 2007;105:562–9
14. Broadhead MW, Kharbanda RK, Peters MJ, MacAllister RJ. KATP channel activation induces ischemic preconditioning of the endothelium in humans in vivo. Circulation 2004;110:2077–82
15. Benjamin N, Calver A, Collier J, Robinson B, Vallance P, Webb D. Measuring forearm blood flow and interpreting the responses to drugs and mediators. Hypertension 1995;25:918–23
16. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Kajiyama G, Oshima T. A noninvasive measurement of reactive hyperemia that can be used to assess resistance artery endothelial function in humans. Am J Cardiol 2001;87:121–5, A9
17. Thijssen DH, Bleeker MW, Smits P, Hopman MT. Reproducibility of blood flow and post-occlusive reactive hyperaemia as measured by venous occlusion plethysmography. Clin Sci (Lond) 2005;108:151–7
18. Tousoulis D, Antoniades C, Stefanadis C. Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. Heart 2005;91:553–8
19. Nawab US, Touch SM, Irwin-Sherman T, Blackson TJ, Greenspan JS, Zhu G, Shaffer TH, Wolfson MR. Heliox attenuates lung inflammation and structural alterations in acute lung injury. Pediatr Pulmonol 2005;40:524–32
20. Englelke KA, Halliwill JR, Proctor DN, Dietz NM, Joyner MJ. Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. J Appl Physiol 1996;81:1807–14
21. Harris PD, Barnes R. The uses of helium and xenon in current clinical practice. Anaesthesia 2008;63:284–93
22. Fink JB. Opportunities and risks of using heliox in your clinical practice. Respir Care 2006;51:651–60
23. Eves ND, Petersen SR, Haykowsky MJ, Wong EY, Jones RL. Helium-hyperoxia, exercise, and respiratory mechanics in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006;174:763–71
24. Pagel PS, Krolikowski JG, Pratt PF Jr., Shim YH, Amour J, Warltier DC, Weihrauch D. Reactive oxygen species and mitochondrial adenosine triphosphate-regulated potassium channels mediate helium-induced preconditioning against myocardial infarction in vivo. J Cardiothorac Vasc Anesth 2008;22:554–9
25. Feng J, Lucchinetti E, Ahuja P, Pasch T, Perriard JC, Zaugg M. Isoflurane postconditioning prevents opening of the mitochondrial permeability transition pore through inhibition of glycogen synthase kinase 3beta. Anesthesiology 2005;103:987–95
26. Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC. Volatile anesthetics mimic cardiac preconditioning by priming the activation of mito KATP channels via multiple signaling pathways. Anesthesiology 2002;97:4–14
27. Carmona M, Lopes RI, Borba M, Omokawa M, Naufal R, Miyaji K, Matsumura N, Vieira N, Pereira PR. Comparison of the effects of carbon dioxide and helium pneumoperitoneum on renal function. J Endourol 2008;22:1077–82
28. Schmeding M, Schwalbach P, Reinshagen S, Autschbach F, Benner A, Kuntz C. Helium pneumoperitoneum reduces tumor recurrence after curative laparoscopic liver resection in rats in a tumor-bearing small animal model. Surg Endosc 2003;17:951–9
29. Brundell SM, Tsopelas C, Chatterton B, Touloumtzoglou J, Hewett PJ. Experimental study of peritoneal blood flow and insufflation pressure during laparoscopy. Br J Surg 2002; 89:617–22
30. Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu Rev Physiol 1995;57:827–72
31. Frenette PS, Denis CV, Weiss L, Jurk K, Subbarao S, Kehrel B, Hartwig JH, Vestweber D, Wagner DD. P-Selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. J Exp Med 2000;191:1413–22
32. Lucchinetti E, Hofer C, Bestmann L, Hersberger M, Feng J, Zhu M, Furrer L, Schaub MC, Tavakoli R, Genoni M, Zollinger A, Zaugg M. Gene regulatory control of myocardial energy metabolism predicts postoperative cardiac function in patients undergoing off-pump coronary artery bypass graft surgery: inhalational versus intravenous anesthetics. Anesthesiology 2007;106:444–57
33. Julier K, da Silva R, Garcia C, Bestmann L, Frascarolo P, Zollinger A, Chassot PG, Schmid ER, Turina MI, von Segesser LK, Pasch T, Spahn DR, Zaugg M. Preconditioning by sevoflurane decreases biochemical markers for myocardial and renal dysfunction in coronary artery bypass graft surgery: a double-blinded, placebo-controlled, multicenter study. Anesthesiology 2003;98:1315–27
34. VanDeripe DR. The swelling of mitochondria from nitrogen gas; a possible cause of reperfusion damage. Med Hypotheses 2004;62:294–6