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Isoflurane–Sevoflurane Administration before Ischemia Attenuates Ischemia–Reperfusion-induced Injury in Isolated Rat Lungs

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Background: **The effects of volatile anesthetics on ischemia–reperfusion (IR)–induced lung injury are not clear. The authors investigated the effects of preadministration of isoflurane and sevoflurane on IR-induced lung injury in an isolated buffer-perfused rat lung model.**

Methods: **Isolated rat lungs were designated into four groups: control group (n = 6): perfusion for 120 min without ischemia; IR group (n = 6): interruption of perfusion and ventilation for 60 min followed by reperfusion for 60 min; sevoflurane (SEVO)-IR (n = 6) and isoflurane (ISO)-IR (n = 6) groups: 1 minimum alveolar concentration (MAC) isoflurane or sevoflurane was administered for 30 min, followed by 60 min ischemia, then 60 min reperfusion. The authors measured the coefficient of filtration (Kfc) of the lung, lactate dehydrogenase (LDH) activity, tumor necrosis factor α, and nitric oxide metabolites (nitrite + nitrate) in the perfusate and the wet-to-dry lung weight ratio.**

Results: **IR caused significant increases in the coefficient of filtration (approximately sevenfold at 60 min of reperfusion compared with baseline; $P < 0.01$), the wet-to-dry lung weight ratio, the rate of increase of lactate dehydrogenase activity, and tumor necrosis factor α in the perfusate, and caused a significant decrease in nitric oxide metabolites in the perfusate. Administration of 1 MAC isoflurane or sevoflurane before ischemia significantly attenuated IR-induced increases in the coefficient of filtration and the wet-to-dry lung weight ratio, inhibited increases in the rate of increase of lactate dehydrogenase activity and tumor necrosis factor α in the perfusate, and abrogated the decrease in nitric oxide metabolites in the perfusate. No difference was found between the SEVO-IR and ISO-IR groups.**

Conclusion: **Isoflurane and sevoflurane administered before ischemia can attenuate IR-induced injury in isolated rat lungs.**
(Key words: Hyperpermeability; isogravimetric; perfusate; weight gain.)

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ISCHEMIA–REPERFUSION (IR)–induced lung injury after cardiac pulmonary bypass or lung transplantation still is a problem in clinical practice. The main underlying disease in IR lung injury is pulmonary vascular endothelial dysfunction manifested by increased vascular permeability that may result in pulmonary edema. Although the mechanisms have not been elucidated completely, recent work indicated that tumor necrosis factor α (TNF-α) is an essential component of the cascade of events that lead to IR-induced lung injury.[1,2] Furthermore, blockade of TNF-α can reduce neutrophil chemotaxis and sequestration and attenuate the lung injury process.[3]

The effects of volatile anesthetics on IR-induced lung injury are not clear. Previous studies have shown that these agents could protect against IR-induced injury of the heart,[4–6] the brain,[7] and the liver.[8] Our previous study showed that isoflurane could attenuate IR-induced injury in rabbit lung.[9] Volatile anesthetics could significantly inhibit the release of TNF-α *in vitro*.[10] These studies suggest that volatile anesthetics may protect against IR-induced lung injury by inhibiting the release of TNF-α. In the current study, we investigated whether administration of volatile anesthetics (isoflurane and sevoflurane) before ischemia could inhibit the release of TNF-α and protect the lung against IR-induced injury in an isolated rat lung model. Because IR-induced lung injury is characterized by increased vascular permeability, we measured the coefficient of filtration (Kfc), an index of vascular permeability, throughout the experiment to assess the integrity of pulmonary vasculature. We also measured the levels of TNF-α, lactate dehydrogenase (LDH) activity, and nitric oxide (NO) metabolites in the perfusate before ischemia and after perfusion.

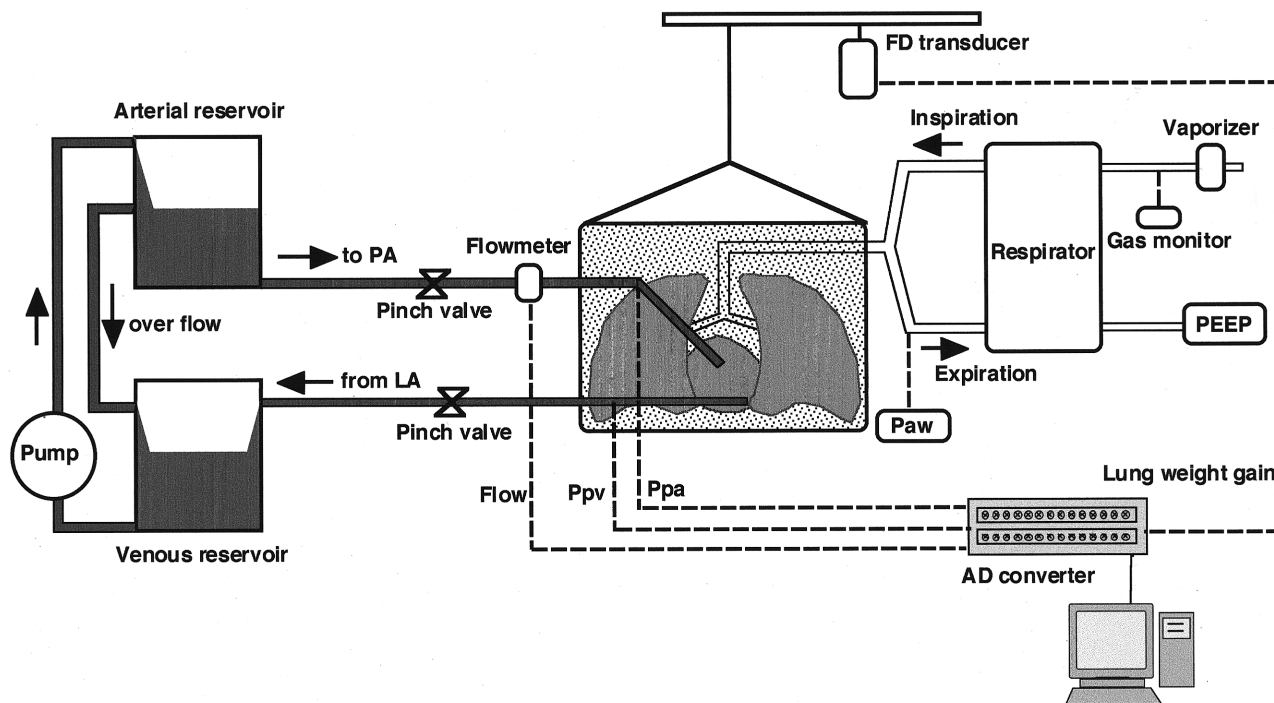

Fig. 1. Schematic presentation of the experimental setup. Arterial and venous reservoirs are elevated simultaneously by 6 cm H_2O during measurement of the coefficient of filtration (Kfc). Two pinch valves are simultaneously occluded for more than 5 s to obtain pulmonary capillary pressure. The pump continuously circulates the perfusate from the venous reservoir to the arterial reservoir to maintain a constant driving pressure. AD = analog-to-digital; FD = force displacement; LA = left atrium; PA = pulmonary artery; P_{aw} = airway pressure; PEEP, positive end-expiratory pressure; Ppa = pulmonary artery pressure; Ppv = pulmonary venous pressure.

Materials and Methods

Isolated Perfused Lung Preparation

The experimental protocol was approved by Tottori University Laboratory Animal Care Committee. Adult male Sprague-Dawley rats (weighing 300–350 g) were anesthetized with pentobarbital (20 mg/kg intraperitoneal) and ketamine (50 mg/kg intramuscular). After local anesthesia with 1.0% lidocaine, tracheostomy and sternotomy were performed. The lungs were mechanically ventilated with a ventilator (model 681; Harvard Apparatus, Natick, MA) using room air at a rate of 50 strokes/min, a tidal volume of 2.5 ml, and a positive end-expiratory pressure (PEEP) of 2 cm H_2O until the cannulation of the pulmonary artery and left atrium was complete, after which the rat was ventilated in the same conditions with a warm humidified gas mixture of 21% O_2, 5% CO_2, and balance nitrogen. After sternotomy, heparin (100 U) was administered *via* the right ventricle and allowed to circulate for approximately 3 min. Catheters were inserted through the right and left ventricles and secured in place with sutures. The lungs were perfused with bicarbonate-buffered physiologic salt solution. Physiologic salt solution contained 119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 22.61 mM $NaHCO_3$, 1.18 mM KH_2PO_4, and 3.2 mM $CaCl_2$. To each 100 ml of this stock solution, we added 100 mg dextrose, 20 mU insulin, and 5 g bovine serum albumin (Sigma, Chemical Co., St. Louis, MO). The pH of perfusate was adjusted to 7.35–7.45 by the addition of sodium bicarbonate. The lungs and heart were removed *en bloc* and suspended from a counterbalanced force-displacement transducer (T1-200-240; Orientec, Tokyo, Japan) into a humidified chamber set at 37°C to monitor weight changes (fig. 1). The first 75 ml perfusate, which contained large amounts of residual blood cells and plasma, was discarded, and an additional 50 ml volume of perfusate was then used for recirculation.

Pulmonary arterial (Ppa) and venous (Ppv) pressures were continuously monitored by transducers connected to amplifiers (model 2238; San-ei, Tokyo, Japan). A flow probe (model FF-050T; Nihon Kohden, Tokyo, Japan) connected to an electromagnetic flowmeter (MFV-3100; Nihon Kohden) was placed in the perfusion circuit for

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continuous monitoring of blood flow (Q). Pulmonary venous pressure was adjusted to 5 mmHg to ensure that pulmonary venous pressure exceeded the mean airway pressure, then pulmonary arterial pressure was adjusted by maintaining the flow at 0.04 ml/g body weight/min by adjustment of the arterial reservoir level at the beginning of the experiment. Consequently, the driving pressure was kept constant throughout the experiment. Zero level for pressures was referenced as the bottom of the lung in the chamber. All signals were digitized using an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster City, CA) and analyzed using a commercially available software (Axogragh version 3.0; Axon Instruments). Isoflurane (Abbott Laboratories, North Chicago, IL) or sevoflurane (Maruishi Pharmaceutical, Osaka, Japan) was administered using isoflurane (Acoma, Tokyo, Japan) or sevoflurane (Penlon; Penlon Limited, Abing, Oxon, UK) vaporizers. The concentration of isoflurane and sevoflurane in inspired gas was monitored using anesthetic agent monitor (Capnomac; Datex Instrumenttarium Corp., Helsinki, Finland).

Total pulmonary vascular resistance (Rt) was calculated using the following formula: Rt = (Ppa − Ppv)/Q.

Coefficient of Filtration

The Kfc was determined using the method described by Drake *et al.*[11] Briefly, after an isogravimetric state was achieved (*i.e.*, no gain or loss of lung weight), the pulmonary capillary pressure (Ppc) was increased by elevating both the arterial and the venous reservoirs by 6 cm H_2O for 7 min, and the lung weight gain was recorded. The initial rapid lung weight gain (caused by vascular filling and distension) was followed by a slower rate of weight gain (caused by capillary filtration). The logarithm of the rate of lung weight gain ($\Delta W/\Delta t$) was plotted as a function of time. We analyzed the $\Delta W/\Delta t$ during a 3- to 6-min interval of increased Ppc. The initial rate of fluid filtration [$(\Delta W/\Delta t)t = 0$] was then calculated by extrapolating $\Delta W/\Delta t$ to time 0. Kfc was calculated by dividing $\Delta W/\Delta t$ at time 0 [$(\Delta W/\Delta t)t = 0$] by changes in Ppc and normalized using the baseline wet lung weight and expressed in $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$ lung tissue.

To determine Ppc, arterial and venous lines both were occluded simultaneously for more than 5 s with fast-pinch valves (PK 0305-NO; Takasago, Tokyo, Japan); pulmonary arterial pressure and pulmonary venous pressure converged to a certain level, which was defined as Ppc. Change in Ppc was calculated as the difference between Ppc measured before and 7 min after elevation of the reservoir level. During Kfc determination, the ventilator was switched off, but a constant flow of 2 l/min of mixed gas was administered at 2 cm H_2O airway pressure. Baseline wet lung weight was estimated at the end of the experiment by subtracting the weight of extrapulmonary tissues from the total weight of the lungs and extrapulmonary tissues, which was measured before perfusion.

Wet-to-dry Weight Ratio

The lungs were used to estimate the tissue wet-to-dry weight ratio. After recording the wet weight of the lung tissue, the lungs were placed in a drying oven at 60°C for 2 weeks and reweighed. Wet-to-dry weight ratio was calculated using the formula: (wet weight − dry weight)/dry weight.

Preparation of Perfusate and Measurement of NO Metabolites, TNF-α, and LDH

Samples of perfusate (2.5 ml each) were obtained immediately after 30 min of equilibration, at 60 min after reperfusion in IR groups, and at these same intervals in the control group. The collected perfusate was centrifuged immediately at 250*g* for 10 min at 4°C. The supernatant was divided into several aliquots and stored at −80°C until analysis. The concentrations of NO metabolites (sum of nitrate and nitrite) were determined using a calorimetric assay kit (Cat#7 80001; Cayman Chemical Company, Ann Arbor, MI).[12] TNF-α level was measured by using the rat TNF-α enzyme-linked immunosorbent assay kit (ELISA; Biosource International, Inc. CA). LDH activity was measured using a modification of the colorimetric method[13] at 37°C. Changes in LDH activity and TNF-α in the perfusate were expressed as rate of increase = (end value − baseline value)/baseline value.

Experimental Protocol

The lungs were allowed to equilibrate for 30 min to achieve an isogravimetric state. Lung preparations were designated into four groups. The total duration of the experiment was similar in each group. In the control group (n = 6), the lungs were continuously perfused and ventilated for 120 min. In the IR group (n = 6), ventilation and perfusion were interrupted (ischemia) after equilibration, and the lungs were maintained for 60 min in the humidified chamber while the airway was kept open. The lungs were reperfused and reventilated for 60 min after ischemia. In the isoflurane (ISO)-IR group (n = 6), 1 minimum alveolar concentration (MAC) isoflurane (1.38%) was first administered for 30 min,

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followed by ischemia for 60 min, and later the preparation was perfused for 60 min. A similar protocol was used in the sevoflurane (SEVO)-IR group (n = 6), except that isoflurane was replaced with 1 MAC sevoflurane (2.11%).

The Kfc was determined at baseline (immediately before ischemia), 30, and 60 min after reperfusion in IR groups and at these same intervals in the control group. Rt was determined at baseline and 60 min after reperfusion in IR groups and at these same intervals in the control group.

Statistical Analysis

Data are presented as the mean ± SD. Within-group differences were analyzed using one-way analysis of variance using repeated measures (Statview 4.5; Abacus Concepts, Berkeley, CA). Multiple samples of the same time intervals were analyzed using one-way analysis of variance. Changes in LDH activity, TNF-α, NO metabolites in the perfusate, and the wet-to-dry weight ratio were evaluated using one-way analysis of variance. The Bonferroni–Dunn test was used for *post hoc* comparisons. Significance was determined when P was less than 0.05.

Results

Changes in Total Vascular Resistance

Total pulmonary vascular resistance significantly increased at the end of the experiment in all groups, compared with baseline, from 279 ± 155 $mmHg \cdot l^{-1} \cdot min^{-1}$ to 381 ± 197 $mmHg \cdot l^{-1} \cdot min^{-1}$ in the control group ($P < 0.05$), from 273 ± 98 $mmHg \cdot l^{-1} \cdot min^{-1}$ to 395 ± 156 $mmHg \cdot l^{-1} \cdot min^{-1}$ in the IR group ($P < 0.05$), from 255 ± 103 $mmHg \cdot l^{-1} \cdot min^{-1}$ to 432 ± 212 $mmHg \cdot l^{-1} \cdot min^{-1}$ in the ISO-IR group ($P < 0.05$), and from 287 ± 119 $mmHg \cdot l^{-1} \cdot min^{-1}$ to 446 ± 147 $mmHg \cdot l^{-1} \cdot min^{-1}$ in the SEVO-IR group ($P < 0.05$). There were no significant differences among groups at both the baseline and the end of the experiment.

Isoflurane and Sevoflurane Protect Against IR-induced Increase in Microvascular Permeability

The level of Kfc (an index of microvascular permeability) at baseline was similar in all groups. Ischemia followed by reperfusion caused a significant increase (twofold at 30 min of reperfusion; $P < 0.01$) in microvascular permeability (Kfc) in the IR group, relative to the control group (fig. 2). Kfc further increased (sevenfold) at 60 min of reperfusion in the IR group ($P < 0.01$). Preadministration of 1 MAC isoflurane or sevoflurane prevented the deterioration in vascular permeability after IR.

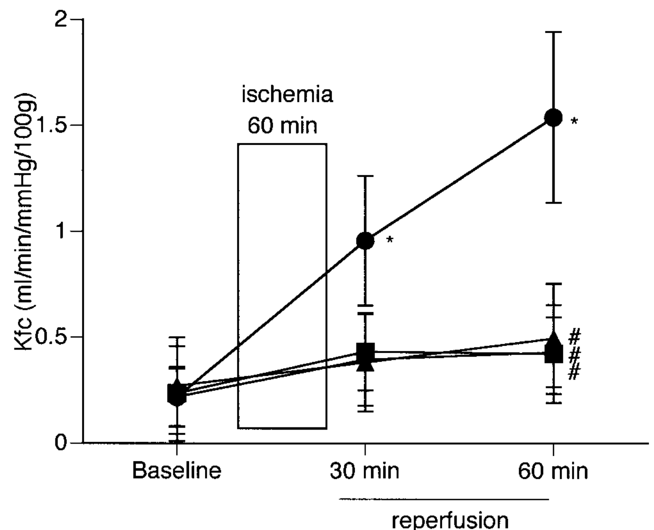

Fig. 2. Changes in the coefficient of filtration (Kfc). Data are mean ± SD (n = 6 per group). Squares = control group; circles = IR group; triangles = ISO-IR group; diamonds = SEVO-IR group. *$P < 0.01$ *versus* baseline. #$P < 0.01$ *versus* IR group at the end of reperfusion.

Isoflurane and Sevoflurane Prevent IR-induced Increase in Lung Wet-to-dry Weight Ratio

The lung wet-to-dry weight ratio was calculated to estimate lung fluid content. IR significantly increased the lung wet-to-dry weight ratio in the IR group. In contrast, preadministration of isoflurane or sevoflurane abrogated the effect of IR on the lung wet-to-dry weight ratio (ISO-IR and SEVO-IR groups; fig. 3).

Isoflurane and Sevoflurane Antagonize IR-induced Changes in LDH, NO, and TNF-α

We used the release of LDH into the perfusate as an estimate of cell damage during the experiment. Figure 4 shows that the rate of increase of LDH activity was significantly higher in the perfusate of the IR group compared with the control group. In contrast, preadministration of isoflurane or sevoflurane significantly inhibited the rate of increase of LDH activity. Measurement of NO metabolites showed a significantly low level of these metabolites in the perfusate at the end of reperfusion in the IR group relative to the control group. In contrast, isoflurane or sevoflurane significantly inhibited the decrease in NO metabolites (ISO-IR and SEVO-IR groups; fig. 5) at the end of reperfusion. The results of evaluation

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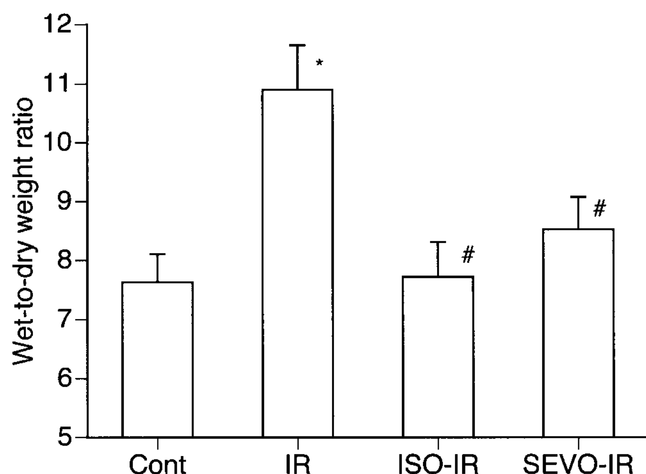

Fig. 3. Changes in lung wet-to-dry weight ratio among groups. Data are mean ± SD (n = 6 per group). *$P < 0.05$ *versus* control group. #$P < 0.01$ *versus* IR group.

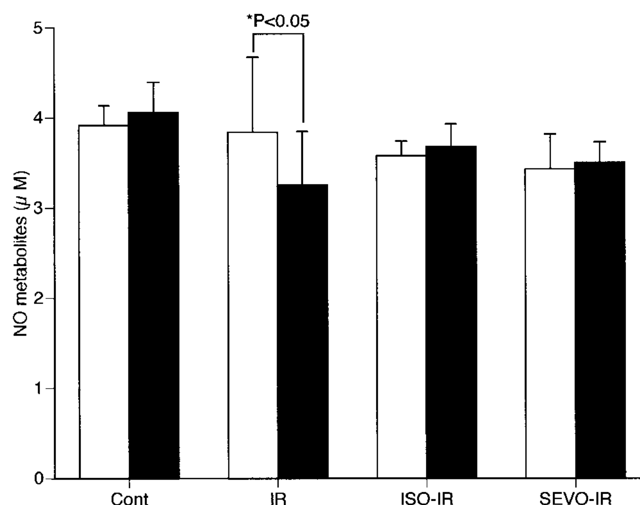

Fig. 5. Changes in nitric oxide (NO) metabolites (sum of nitrite and nitrate) in the perfusate. Data are mean ± SD (n = 6 per group). (Open bars) Baseline level; (closed bars) NO metabolite level at the end of reperfusion.

of the potential mechanism of the protective effects of isoflurane or sevoflurane against IR lung injury are shown in figure 6. IR significantly increased the rate of increase of TNF-α release in the IR group compared with the control group. Preadministration of isoflurane or sevoflurane significantly inhibited the rate of increase of TNF-α in the perfusate.

Discussion

The main finding of the current study was that isoflurane and sevoflurane protected against lung injury induced by IR in an isolated rat lung model. Therefore, administration of 1 MAC isoflurane or sevoflurane before ischemia significantly attenuated the increase in Kfc (fig. 2) and wet-to-dry weight ratio (fig. 3), inhibited the release of LDH (fig. 4) and TNF-α (fig. 6), and reduced the production of NO metabolites (fig. 5) in the perfusate after IR.

The protective effect of volatile anesthetics against IR-induced injury has been described previously in other important organs (heart, brain, among others) when the volatile anesthetics were administered before ischemia[4,7] or during early reperfusion,[5] or when both were

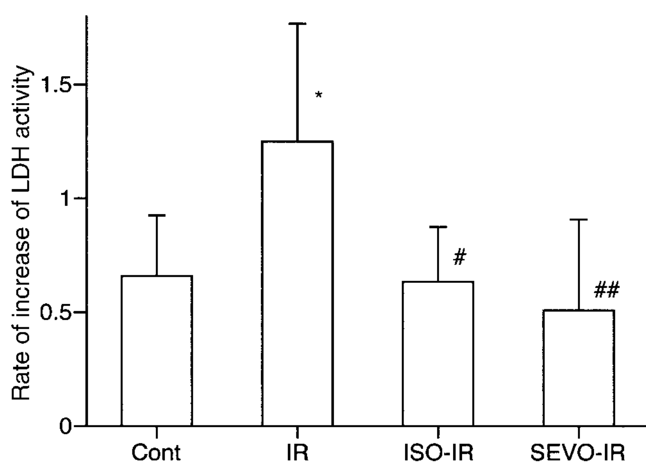

Fig. 4. Changes in rate of increase of lactate dehydrogenase (LDH) activity in the perfusate among groups. Data are mean ± SD (n = 6 per group). *$P < 0.05$ *versus* control group. #$P < 0.05$ *versus* IR group. ##$P < 0.01$ *versus* IR group.

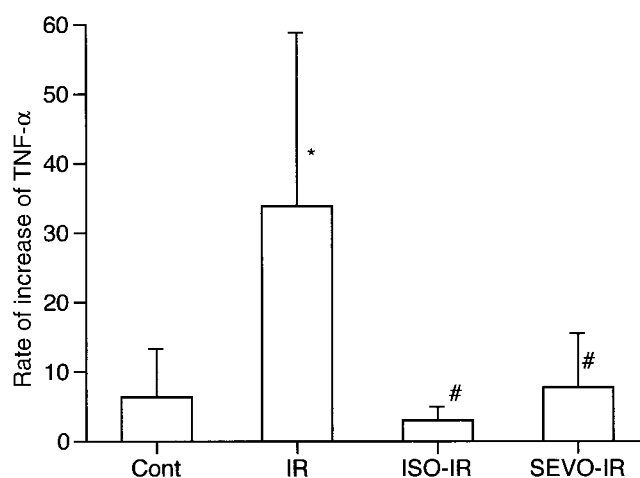

Fig. 6. Changes in rate of increase of tumor necrosis factor α (TNF-α) in the perfusate among groups. Data are mean ± SD (n = 6 per group). *$P < 0.05$ *versus* control group. #$P < 0.05$ *versus* IR group.

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administered before ischemia and during reperfusion.[6] In isolated blood-free perfused livers, isoflurane, administered during ischemia and reperfusion, attenuated hypoxia–reoxygenation injury in rats.[8] Isoflurane, similar to halothane, administered before, during, and immediately after hypothermic preservation markedly improved cardiac perfusion and function.[14] Sevoflurane, a new volatile anesthetic used clinically in many centers, also protects the heart and brain against IR-induced injury.[6,15]

Although the effects of volatile anesthetics on IR-induced lung injury remain unclear, in a mouse model of multiple organ dysfunction syndrome, isoflurane (1.5%) attenuated lung inflammation and injury, probably by modulating the inflammatory response.[16] Halothane and isoflurane could protect against oxidant injury in aortic endothelial cells by amelioration of the toxic increase in cytosolic calcium.[17] In an isolated rabbit lung model, isoflurane could attenuate IR-induced injury.[9] In contrast to these findings, Nielsen *et al.*[18,19] recently provided evidence that indicated that volatile anesthetics (halothane[18] and desflurane[19]) have detrimental effects on IR injury in the liver[18] and lung[19] induced by thoracic aorta occlusion–reperfusion in a rabbit model. Although no differences in lung wet-to-dry ratio were found among groups, the findings of Nielsen *et al.*[19] raised an important issue regarding the effects of volatile anesthetics on IR-mediated lung injury. These studies are, however, not directly comparable with ours because of differences in the experimental design in which an *in vivo* animal model was used and the lung injury was induced by reperfusion of the liver and intestines by thoracic aorta occlusion–reperfusion. The differences in the study design and the use of different models may have contributed to the observed differences in the results.

In contrast with our previous study in the rabbit,[9] a different anesthetic (sevoflurane), species (rat), and lung injury indexes (LDH, NO metabolites) were used, and the possible mechanism (role of TNF-α) were investigated in the current study. IR lung injury, directly induced by IR of the isolated lungs, was evident by the increased Kfc and wet-to-dry weight ratio and the changes in LDH activity and NO metabolites in the perfusate. Kfc measured during isogravimetric conditions is a reliable and reproducible measure of lung injury in isolated animal lungs.[11,20–22] A high Kfc means augmented microvascular permeability. In the current study, the results of Kfc were consistent with those of the wet-to-dry weight ratio, which indicates that isoflurane and sevoflurane could inhibit IR-induced increase in vascular permeability. Our findings of changes in LDH activity and NO metabolites in the perfusate further confirm that IR lung injury was induced in our *in vitro* model. Reduced levels of NO metabolites in the perfusate noted in the current study reflected a low production of NO, which was probably a result of dysfunction of pulmonary endothelial cells caused by IR. Administration of isoflurane or sevoflurane before ischemia successfully protected the lung from IR injury, as indicated by the Kfc, the wet-to-dry weight ratio, LDH activity, and the NO metabolites in the perfusate.

As discussed previously, we demonstrated that volatile anesthetics reduce lung injury. However, whether this is because of prevention of changes or whether the volatile anesthetic itself produces some off-setting decrease in these markers is not clear. It is possible that the effect of volatile anesthetics is the opposite of that of IR, resulting in a net benefit, rather than being caused by a specific inhibition of certain pathologic processes. To confirm this conclusion, a supplementary experiment in which control lungs are ventilated with volatile anesthetic but not subjected to ischemia is necessary. We performed such experiment by administration of 1 MAC isoflurane followed by 120 min perfusion (ISO-control group, n = 5). No significant difference in currently used markers was found between the ISO-control group and the control group. In the ISO-control, Kfc was 0.39 ± 0.23 ml $\cdot$ min^{-1} $\cdot$ mmHg^{-1} $\cdot$ 100 g^{-1} at baseline and 0.45 ± 0.15 ml $\cdot$ min^{-1} $\cdot$ mmHg^{-1} $\cdot$ 100 g^{-1} at the end of perfusion ($P = 0.733$). Wet-to-dry ratio was 7.00 ± 0.97. The rates of increase of LDH activity and TNF-α were 0.58 ± 0.21 and 9.50 ± 5.25, respectively. The sum of NO metabolites in the perfusate was 3.93 ± 0.16 μM at baseline and 4.07 ± 0.34 μM at the end of perfusion ($P = 0.067$). These results showed that the volatile anesthetic itself does not produce any off-setting decrease in the currently used markers.

Although the precise mechanisms of IR-induced organ injury are still being investigated, recent studies indicate that TNF-α is an essential component in some models of lung injury.[1,23] TNF-α was significantly released after IR. Furthermore, the damage associated with IR was totally eliminated by pretreating animals with a specific anti–TNF-α antibody.[1] Significant elevation of local tissue levels of TNF-α was also observed after reperfusion after prolonged pulmonary ischemia.[24] TNF up-regulates intercellular adhesion molecule 1 (ICAM-1), which is important in neutrophil-dependent lung and liver injury associated with hepatic IR in the rat.[2] In the current study, the rate of increase of TNF-α in the perfusate was significantly increased after IR, whereas preadministra-

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tion of isoflurane or sevoflurane significantly inhibited such an increase. These results suggest that the protective effect of isoflurane and sevoflurane against IR-induced lung injury may be mediated through inhibition of TNF-α release. Previous studies reported that volatile anesthetics could reduce postischemic adhesion of neutrophils in the coronary system in an IR heart model,[25] inhibit neutrophil migration[26] and generation of oxygen radicals by inflammatory cells,[27] and inhibit the release of TNF-α from cultured human peripheral mononuclear cells.[10] However, because our rat model is devoid of circulating neutrophils and free of specific markers of neutrophil activation, it is difficult to discern the source of TNF-α; however, it is possible that the resident neutrophils may be activated by the endothelial elaboration of TNF-α after ischemia or cause further elaboration of TNF-α by further injury of the reperfused endothelium. Considered together, further studies are warranted to investigate the exact role of TNF-α.

Considering that the volatile anesthetics are also excellent pulmonary vasodilators, the observed protective effect may represent changes secondary to changes in vascular smooth muscle tone; but no significant Rt difference was found among the groups at baseline or the end of the experiment in the current buffer-perfused lung model. Other possible explanations for the protective effects of isoflurane and sevoflurane on IR-induced lung injury include suppression of metabolism or depression of the use of adenosine triphosphate (ATP), and activation of potassium adenosine triphosphate channels. Isoflurane can decrease oxygen consumption in the hypoxia-reoxygenated liver[28] and directly preconditions the myocardium against infarction *via* activation of potassium adenosine triphosphate channels.[29] Further studies are warranted.

The main shortcoming of the current study is the lack of any histologic evidence, which confirms the presence of lung injury. Although the isolated and perfused lung preparation is a suitable model for investigating the pulmonary response to injury and other pathophysiologic conditions, such an isolated lung model does not show many features associated with actual lung injury, and the artificial features also make it difficult to extrapolate the findings to the clinical situation. As we noticed in the current study, Rt significantly increased in all groups at the end of the experiments. Furthermore, the effects of potentially toxic metabolites of volatile anesthetics could not be evaluated in the isolated lung model because isoflurane and sevoflurane are primarily metabolized in the liver. Further studies using *in vivo* experimental preparations, in addition to clinical studies, are warranted.

In conclusion, we demonstrated that isoflurane and sevoflurane, when administered before ischemia, exhibit beneficial effects manifested by attenuation of IR injury in the isolated perfused rat lungs.

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References

1. Khimenko P, Bagby G, Fuseler J, Taylor A: Tumor necrosis factor-alpha in ischemia and reperfusion injury in rat lung. J Appl Physiol 1998; 85:2005–11

2. Colletti L, Cortis A, Lukacs N, Kunkel S, Green M, Strieter R: Tumor necrosis factor up-regulates intercellular adhesion molecule 1, which is important in the neutrophil-dependent lung and liver injury associated with hepatic ischemia and reperfusion in the rat. Shock 1998; 10:182–91

3. Tassiopoulos A, Hakim T, Finck C, Pedoto A, Hodell M, Landas S, McGraw D: Neutrophil sequestration in the lung following acute aortic occlusion starts during ischaemia and can be attenuated by tumour necrosis factor and nitric oxide blockade. Eur J Vasc Endovasc Surg 1998; 16:36–42

4. Cope D, Impastato W, Cohen M, Downey J: Volatile anesthetics protect the ischemic rabbit myocardium from infarction. ANESTHESIOLOGY 1997; 86:699–709

5. Preckel B, Schlack W, Thamer V: Enflurane and isoflurane, but not halothane, protect against myocardial reperfusion injury after cardioplegic arrest with HTK solution in the isolated rat heart. Anesth Analg 1998; 87:1221–7

6. Mathur S, Karmazyn M: Interaction between anesthetics and the sodium-hydrogen exchange inhibitor HOE 642 (cariporide) in ischemic and reperfused rat hearts. ANESTHESIOLOGY 1997; 87:1460–9

7. Patel P, Drummond J, Cole D, Kelly P, Watson M: Isoflurane and pentobarbital reduce the frequency of transient ischemic depolarizations during focal ischemia in rats. Anesth Analg 1998; 86:773–80

8. Kon S, Imai M, Inaba H: Isoflurane attenuates early neutrophil-independent hypoxia-reoxygenation injuries in the reperfused liver in fasted rats. ANESTHESIOLOGY 1997; 86:128–36

9. Liu R, Ishibe Y, Ueda M, Hang Y: Isoflurane administration before ischemia and during reperfusion attenuates ischemia/reperfusion-induced injury of isolated rabbit lungs. Anesth Analg 1999; 89:561–5

10. Mitsuhata H, Shimizu R, Yokoyama M: Suppressive effects of volatile anesthetics on cytokine release in human peripheral blood mononuclear cells. Int J Immunopharmacol 1995; 17:529–34

11. Drake R, Gaar K, Taylor A: Estimation of the filtration coefficient of pulmonary exchange vessel. Am J Physiol 1978; 234:H266–74

12. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982; 126:131–8

13. Beauchamp C, Fridovich I: Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971; 44:276–87

14. Stowe D, Fujita S, Bosnjak Z: Improved contractility and coronary flow in isolated hearts after 1-day hypothermic preservation with

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isoflurane is not dependent on K(ATP) channel activation. ANESTHESIOLOGY 1998; 81:233–44

15. Nakajima Y, Moriwaki G, Ikeda K, Fujise Y: The effects of sevoflurane on recovery of brain energy metabolism after cerebral ischemia in the rat: A comparison with isoflurane and halothane. Anesth Analg 1997; 85:593–9

16. Shayevitz J, Varani J, Ward P, Knight P: Halothane and isoflurane increase pulmonary artery endothelial cell sensitivity to oxidant-mediated injury. ANESTHESIOLOGY 1991; 74:1067–77

17. Johnson ME, Sill JC, Uhl CB, Halsey TJ, Gores GJ: Effect of volatile anesthetics on hydrogen peroxide-induced injury in aortic and pulmonary arterial endothelial cells. ANESTHESIOLOGY 1996; 84:103–16

18. Nielsen VG, Tan S, Kirk KA, Baird MS, McCammon AT, Samuelson PN, Parks DA: Halothane and xanthine oxidase increase hepatocellular enzyme release and circulating lactate after ischemia-reperfusion in rabbits. ANESTHESIOLOGY 1997; 87:908–17

19. Nielsen V, Baird M, McAdams M, Freeman B: Desflurane increases pulmonary alveolar-capillary membrane permeability after aortic occlusion-reperfusion in rabbits: Evidence of oxidant-mediated lung injury. ANESTHESIOLOGY 1998; 88:1524–34

20. Miyahara T, Shibamoto T, Wang H, Koizumi T, Honda T, Kubo K, Sekiguchi M, Koyama S: Lecithinized superoxide dismutase attenuates phorbol myristate acetate-induced injury in isolated dog lung. Eur J Pharmacol 1998; 344:231–9

21. Moore T, Khimenko P, Taylor A: Endothelial damage caused by ischemia and reperfusion and different ventilatory strategies in the lung. Chin J Physiol 1996; 39:65–81

22. Parker J, Ivey C, Tucker J: Gadolinium prevents high airway pressure-induced permeability increases in isolated rat lungs. J Appl Physiol 1998; 84:1113–8

23. Chiang C, Hsu K, Yan H, Harn H, Chang D: PGE1, dexamethasone, U-74389G, or Bt2-cAMP as an additive to promote protection by UW solution in I/R injury. J Appl Physiol 1997; 83:583–90

24. Abolhoda A, Brooks A, Choudhry M, Kaneda Y, Liu D, Cheng H, Burt M: Characterization of local inflammatory response in an isolated lung perfusion model. Ann Surg Oncol 1998; 5:87–92

25. Kowalski C, Zahler S, Becker B, Flaucher A, Conzen P, Gerlach E, Peter K: Halothane, isoflurane, and sevoflurane reduce postischemic adhesion of neutrophils in the coronary system. ANESTHESIOLOGY 1997; 86:188–95

26. Tait A, Davidson B, Johnson K, Remick D, Knight P: Halothane inhibits the intraalveolar recruitment of neutrophils, lymphocytes, and macrophages in response to influenza virus infection in mice. Anesth Analg 1993; 76:1106–13

27. Barth J, Petermann W, Entzian P, Wustrow C, Wustrow J, Ohnhaus E: Modulation of oxygen-free radicals from human leukocytes during halothane- and enflurane-induced general anesthesia. Acta Anaesthesiol Scand 1987; 31:740–3

28. Araki M, Inaba H, Mizuguchi T: Isoflurane modulates phorbol myristate acetate-, prostaglandin D2-, and prostaglandin E2-induced alterations in hepatic flow and metabolism in the perfused liver in fasted rats. Anesth Analg 1994; 79:267–73

29. Kersten J, Schmeling T, Pagel P, Gross G, Warltier D: Isoflurane mimics ischemic preconditioning via activation of K(ATP) channels: Reduction of myocardial infarct size with an acute memory phase. ANESTHESIOLOGY 1997; 87:361–70

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