Desflurane Increases Pulmonary Alveolar–
Capillary Membrane Permeability after Aortic
Occlusion–Reperfusion in Rabbits

Evidence of Oxidant-mediated Lung Injury

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Background: Pulmonary injury occurs after vascular surgery, with xanthine oxidase (an oxidant generator) released from reperfusing liver and intestines mediating a significant component of this injury. Because halogenated anesthetics have been observed to enhance oxidant-mediated injury in vitro, the authors hypothesized that desflurane would increase alveolar–capillary membrane permeability mediated by circulating xanthine oxidase after thoracic occlusion and reperfusion.

Methods: Rabbits were assigned to one of five groups: aorta occlusion groups administered desflurane (n = 14), desflurane and tungstate (xanthine oxidase inactivator, n = 12), fentanyl plus droperidol (n = 13), and two sham-operated groups (desflurane, n = 7 and fentanyl plus droperidol, n = 7). Aortic occlusion was maintained for 45 min with a balloon catheter, followed by 3 h of reperfusion. Alveolar–capillary membrane permeability was assessed by measurement of bronchoalveolar lavage fluid protein. Xanthine oxidase activity was determined in plasma and lung tissue. Ascorbic acid content (an antioxidant) was determined in lung tissue.

Results: Desflurane was associated with significantly increased alveolar–capillary membrane permeability after aortic occlusion–reperfusion when compared with the fentanyl plus droperidol anesthesia or sham-operated groups (P < 0.05). Inactivation of xanthine oxidase abrogated the alveolar–capillary membrane compromise associated with desflurane. Although significantly greater than for sham-operated animals, plasma xanthine oxidase activities released after aortic occlusion–reperfusion were not different between the two anesthetics. There were no anesthetic-associated differences in lung tissue xanthine oxidase activity. However, desflurane anesthesia resulted in a significant reduction in lung ascorbic acid after aortic occlusion–reperfusion compared with the sham-operated animals.

Conclusions: Desflurane anesthesia increased xanthine oxidase-dependent alveolar–capillary membrane compromise after aortic occlusion–reperfusion in concert with depletion of a key tissue antioxidant. (Key words: Desflurane; enzymes; ischemia; oxidants; shock; volatile anesthetics; xanthine oxidoreductase.)

PULMONARY injury often occurs after trauma and major vascular surgery. 1-3 Hepatoenteric ischemia commonly occurs in these settings. Although age and concurrent organ dysfunction increase the risk of postoperative respiratory failure after hepatoenteric ischemia, 1,2 the release/activation of circulating plasma oxidant generators after hepatoenteric ischemia–reperfusion have been observed to play an important role in activating a cascade of inflammatory events. 4-9 Indeed, the liver and intestine of most mammalian species contain the highest tissue-specific activity of the oxidant-generating enzyme, xanthine oxidoreductase (EC 1.2.3.2), that displays both oxidase and dehydrogenase activities. 10 Xanthine oxidase, in the presence of purine substrate, reduces molecular oxygen to the reactive species...
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superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and in the presence of metals, hydroxyl radical (·OH).$^{10-15}$ Both forms of the enzyme are called xanthine oxidase, because release of the dehydrogenase form into plasma results in immediate conversion to the oxidase.$^{14}$ In addition to experiencing ischemia and subsequent oxidant stress, after trauma and vascular surgery patients often require a general anesthetic for therapeutic and diagnostic procedures. A halogenated anesthetic agent is usually administered in these settings.$^{15-17}$ Although it is generally accepted that oxidants play a role in lung injury after hepatotenteric ischemia–reperfusion, the mitigating influence of specific anesthetic agents is not well defined.

Halogenated anesthetics have been shown to increase oxidant-mediated pulmonary endothelial cell injury in vitro$^{18}$ and lung injury ex vivo.$^{19}$ In contrast, both isoflurane and halothane decrease H$_2$O$_2$-mediated aortic endothelial cell injury in vitro.$^{20}$ However, neither anesthetic agent protected pulmonary endothelial cells from oxidant stress.$^{20}$ These studies serve as the rational basis for defining in vivo the pulmonary response to an oxidative stress occurring in response to surgical intervention, as well as the mitigating influence of halogenated anesthetic agents.

The purpose of the current study was to determine if desflurane exacerbates or attenuates pulmonary alveolar–capillary membrane permeability in a rabbit model of thoracic aorta occlusion–reperfusion compared with an intravenous anesthetic (fentanyl plus droperidol). We hypothesized that desflurane administration could increase pulmonary alveolar–capillary membrane permeability by increasing the release of xanthine oxidase activity from reperfusing liver and intestines or by modulating the toxicity of xanthine oxidase to the lung after aortic occlusion–reperfusion.

Materials and Methods

The study was approved by the Animal Review Committee of the University of Alabama at Birmingham. All animals received humane care in compliance with the Principles of Laboratory Care formulated by the National Society for Medical Research and with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication no. 86-23, revised 1985, U.S. Government Printing Office, Washington, DC).

Experimental Groups

Male, New Zealand White rabbits (Myrtle’s Rabbis, Thompson Station, TN) that weighed 2–3 kg were fed a standard diet (n = 41) or a molybdenum-deficient, 0.07% w/w sodium tungstate–enriched diet (Purina Mills, St. Louis, MO) (n = 12) for 10 days before experimentation. Previous investigations showed that administration of sodium tungstate results in a significant reduction in tissue and plasma xanthine oxidase activity (<10% of normal activity) in rabbits.$^9$ The animals were then fasted for 14–16 h before experimentation but allowed free access to water.

All rabbits were anesthetized with 10 mg/kg intravenous ketamine (Parke-Davis, Morris Plains, NJ) via a marginal ear vein. Animals were assigned randomly to one of the following maintenance anesthetic regimens: (1) 75 µg·kg$^{-1}$·h$^{-1}$ intravenous fentanyl (Elkins-Sinn, Cherry Hill, NJ) and 3.75 mg·kg$^{-1}$·h$^{-1}$ intravenous droperidol (American Reagent Laboratories, Shirley, NY) via a marginal ear vein, or (2) inhaled 9% desflurane (Ohmeda Pharmaceutical Products Division, Liberty Corner, NJ). All animals were administered 40% oxygen, balance nitrogen. The inspired concentration of oxygen was monitored with an airway gas monitor (model 254; Datex, Helsinki, Finland) that was calibrated daily. Desflurane administration (inspired concentration) was monitored with an anesthetic agent monitor (model 8100; BCI International, Waukesha, WI) that was calibrated daily. After induction of anesthesia, incision sites were infiltrated subcutaneously with 1% lidocaine for additional analgesia.

Rabbits were also assigned randomly to undergo sham operation (sham) or aortic occlusion–reperfusion (aortic occlusion), or aortic occlusion–reperfusion after xanthine oxidase inactivation with sodium tungstate. Consequently, there were a total of five groups: (1) sham, fentanyl plus droperidol (n = 7); (2) aortic occlusion, fentanyl plus droperidol (n = 14); (3) sham, desflurane (n = 7); (4) aortic occlusion, desflurane (n = 14); and (5) aortic occlusion, desflurane, sodium tungstate (n = 12).

Surgical Protocol

Arterial pressure was monitored by placement of a 22-gauge central ear artery catheter and a right femoral arterial catheter. After tracheostomy, mechanical ventilation (fraction of inspiratory oxygen = 0.4) was performed with the partial pressure of carbon dioxide maintained at 32–45 mmHg (4.2–6 kPa). Pancuronium bromide (Elkins-Sinn) was given at a rate of 0.1

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mg·kg$^{-1}$·h$^{-1}$ to ensure relaxed chest wall muscle tone and to minimize barotrauma. The peak inspiratory pressure was also kept between 10−15 mmHg throughout the study to standardize the confounding effects of barotrauma. Central venous access was obtained via the right internal jugular vein for pressure monitoring and fluid administration. All pressures were recorded on a Grass model 7D polygraph (Grass Instruments, Quincy, MA). All rabbits received a maintenance infusion of lactated Ringer’s at 20 ml·kg$^{-1}$·h$^{-1}$, and esophageal temperatures were maintained at 38 or 39°C with a heating pad. A 30-min equilibration period followed completion of the surgical preparation.

**Aortic Occlusion–Reperfusion Protocol**

Sham-operated animals had the left femoral artery exposed, with sham aortic occlusion beginning with ligation of the femoral artery. The aortic occlusion groups also underwent a left femoral cutdown, with insertion of a 4-French Fogarty embolectomy catheter (American Edwards Laboratory, Irvine, CA) into the thoracic aorta with the balloon placed 1 or 2 cm above the diaphragm, as confirmed by postmortem examination. Aortic occlusion was achieved by inflating the catheter balloon with saline. A femoral arterial pressure of 0−10 mmHg confirmed subdiaphragmatic ischemia. After 45 min of occlusion, the balloon was deflated and the catheter removed. Reperfusion was verified by return of pulsatile flow to the femoral arterial line and transient hypotension as measured by the car arterial line. Postoclusion shock was treated according to the algorithms here in Resuscitation Protocol. Arterial blood samples were removed at 180 min of reperfusion. The blood was centrifuged and the plasma assayed for lactate dehydrogenase (LDH) and xanthine oxidase activity, as described in Biochemical Analyses. The release of LDH activity served as a marker of systemic injury. Circulating xanthine oxidase activity was determined because it was shown previously to contribute to permeability pulmonary edema after hepatoenteric ischemia–reperfusion. After 3 h of reperfusion, the rabbits were killed with an overdose of pentobarbital (65 mg/kg).

**Resuscitation Protocol**

Fluids and medications were administered with an Omni-Flow 4000 infusion pump system (Abbott Laboratories, North Chicago, IL). Resuscitation was administered as follows.

**Lactated Ringer’s Administration.** At the beginning of reperfusion, a bolus (20 ml/kg) of lactated Ringer’s solution was administered over 2 min and the infusion rate was adjusted to maintain central venous pressure at the 30-min equilibration value ± 2 mmHg.

**Phenylephrine Administration.** Phenylephrine (Elkins-Sinn) administration began at reperfusion and was adjusted as follows: If the central venous pressure equaled the 30-min equilibration value ± 2 mmHg and the mean arterial blood pressure was <85% of the 30-min equilibration value, phenylephrine was administered.

**Sodium Bicarbonate Administration.** Sodium bicarbonate 8.4% (Abbott Laboratories) was infused intravenously to maintain the arterial base excess near zero.

**Bronchoalveolar Lavage Analyses**

At the end of each experiment, the lungs were removed and the mainstem bronchus ligated. The right lung was lavaged with 20-ml aliquots of 0.9% saline until 50 ml of bronchoalveolar lavage was obtained. The lavage was centrifuged at 1,000g for 10 min. The protein concentration of the cell-free supernatant was determined as a marker of pulmonary alveolar-capillary membrane compromise. The LDH activity of the supernatant served as a marker of alveolar-capillary membrane compromise and alveolar cell lysis.

**Tissue Analyses**

Tissue biopsies were obtained from the upper and lower lobes of the left lung. The left upper lobe was used to obtain the tissue wet-to-dry weight ratio. After recording the wet weight of the tissue sample, it was placed in a drying oven at 70°C for 2 weeks and reweighed. An increase in the ratio of lung wet-to-dry weight ratio served as a gross measure of tissue edema. The left lower lobe was used to assess (1) accumulation of net tissue xanthine oxidase activity as a source of increased oxidant stress and (2) depletion of tissue ascorbic acid as a measure of oxidant stress. The left lower lobe was placed in a preweighed centrifuge tube containing 5 ml of 50 mm potassium phosphate buffer at pH 7.4 with protease inhibitors (2 μg/ml leupeptin, 0.5 mg/ml Pefabloc® (Boehringer Mannheim, Mannheim, Germany), and 0.1 mm EDTA and subsequently homogenized on ice. The homogenate was centrifuged at 40,000g at 4°C for 30 min. The supernatant was decanted and stored at −85°C before assay.

**Biochemical Analyses**

Lung tissue samples were stored at −85°C before determination of ascorbic plus dehydroascorbic acid by a

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modification of a fluorometric method. The LDH activity of fresh plasma and bronchoalveolar lavage samples was measured according to a modification of a spectrophotometric method22 at 37°C. Plasma and bronchoalveolar lavage fluid samples were assayed for total protein concentration by a modification of a spectrophotometric method.23

Plasma and lung tissue samples were prepared and processed as previously. Briefly, samples were subjected to size-exclusion chromatography with a G-25 column (Pharmacia, Piscataway, NJ) to remove low molecular weight inhibitors of xanthine oxidase. Xanthine oxidase activity was determined from the rate of production of uric acid in the presence of xanthine (75 μM) and nicotinamide adenine dinucleotide (0.5 mM) by incubation for 60 min at 37°C. One unit of activity (U) is defined as 1 μmol/min urate formed at 37°C and pH 7.4. Allopurinol (100 μM), an inhibitor of xanthine oxidase, was used in parallel samples to confirm that urate formation was specifically due to xanthine oxidase activity. Oxonic acid (0.01 mM) was added to all samples to inhibit uricase, an enzyme found in rabbits that oxidizes urate to allantoin, thus preventing underestimation of rates of product formation by xanthine oxidase. Reactions were terminated by deproteinization with acetonitrile, and the uric acid content of samples were determined using a high-performance liquid chromatographic-based electrochemical technique.24

Xanthine oxidase activity was determined in the following in vitro experiments spectrophotometrically at 295 nm (ε = 1.1 · 10^3 M^-1 cm^-1) by the rate of uric acid production, as previously described.25

Analysis of the Effects of Anesthetics on Xanthine Oxidase Activity

To determine the influence of fentanyl plus droperidol and halogenated anesthetics on lung injury via direct effects on xanthine oxidase catalytic activity, in vitro analyses of anesthetic-containing buffers were performed. Xanthine oxidase (Calbiochem, La Jolla, CA) was desalted by chromatography on a sephadex G-25 column having a 10-ml bed volume (Pharmacia Biotech, Piscataway, NJ) by elution with 50 mm potassium phosphate, pH 7.4. Potassium phosphate buffer (50 mm, pH 7.4) saturated with desflurane was prepared by bubbling 9% desflurane carried in air (21% oxygen, 79% nitrogen, 5 l/min for 2 min) through 60 ml of buffer. Xanthine (final concentration, 100 μM) and xanthine oxidase (final concentration, 5 μM/ml) were added to reactions saturated as described before with desflurane in a closed cuvette using gas-tight solution delivery techniques. Xanthine oxidase (final concentration, 5 mU/ml) was diluted in a solution containing 100 μM xanthine, 50 mm potassium phosphate buffer (pH 7.4), and 2.5% fentanyl plus droperidol. The concentration of fentanyl plus droperidol used in vitro was equivalent to the expected in vivo plasma concentration if approximately one half of the typical hourly dose was administered to the rabbits as a bolus injection. Xanthine oxidase activity was determined spectrophotometrically at 295 nm, as previously mentioned.25

Statistical Analysis

All variables are expressed as means ± SD. Analysis of the effect of anesthesia on all measured parameters in the sham-operated animals was performed with repeated-measures analysis of variance (ANOVA) or the Student's t test. If no significant differences were found, the values of the two sham-operated groups were combined for all analyses comparing the different aortic occlusion groups. Analysis of the effect of anesthesia, aortic occlusion-reperfusion, and xanthine oxidase inactivation on hemodynamic variables was performed with repeated-measures ANOVA. Analysis of the effect of anesthesia, aortic occlusion-reperfusion, and xanthine oxidase inactivation on the partial pressure of oxygen (Pao2) was conducted by two-way ANOVA. Analysis of the effect of anesthesia and aortic occlusion-reperfusion on bronchoalveolar lavage protein concentration, bronchoalveolar lavage LDH activity, lung tissue xanthine oxidase activity, lung tissue ascorbic acid content, and lung wet-to-dry weight ratio was conducted by one-way measures ANOVA. Analysis of the effect of anesthesia and aortic occlusion-reperfusion on plasma LDH activity, xanthine oxidase activity, and protein concentration was conducted by one-way ANOVA. Analysis of the effect of xanthine oxidase inactivation and aortic occlusion-reperfusion on bronchoalveolar lavage protein concentration, bronchoalveolar lavage LDH activity, lung tissue xanthine oxidase activity, lung tissue ascorbic acid content, lung wet-to-dry weight ratio, plasma LDH activity, plasma xanthine oxidase activity, and plasma protein content was conducted by one-way ANOVA. The Student-Newman-Keuls test was used for post hoc comparisons. Analysis of the effects of anesthetics and xanthine oxidase inactivation on resuscitation requirements (e.g., lactated Ringer's solution) after aortic occlusion-reperfusion was performed using the Student's t test. Analysis of the effects of anesthetics on xanthine oxidase activity in vitro was performed by

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Table 1. Mean Arterial (MAP) and Central Venous (CVP) Pressures for All Groups (mmHg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pressure</th>
<th>30 EQ</th>
<th>45 AO</th>
<th>60 R</th>
<th>120 R</th>
<th>180 R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>MAP</td>
<td>80 ± 17</td>
<td>76 ± 17</td>
<td>72 ± 12</td>
<td>72 ± 9</td>
<td>71 ± 10</td>
</tr>
<tr>
<td></td>
<td>CVP</td>
<td>3 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 1</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Aortic occlusion + fentanyl + droperidol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>74 ± 9</td>
<td>89 ± 11</td>
<td>62 ± 9</td>
<td>70 ± 7</td>
<td>75 ± 9</td>
</tr>
<tr>
<td></td>
<td>CVP</td>
<td>3 ± 2</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>4 ± 2</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Aortic occlusion + desflurane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>83 ± 13</td>
<td>79 ± 13</td>
<td>75 ± 14</td>
<td>77 ± 15</td>
<td>79 ± 10</td>
</tr>
<tr>
<td></td>
<td>CVP</td>
<td>2 ± 1</td>
<td>3 ± 2</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Aortic occlusion + desflurane + sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tungstate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>78 ± 13</td>
<td>78 ± 14</td>
<td>74 ± 11</td>
<td>78 ± 9</td>
<td>80 ± 7</td>
</tr>
<tr>
<td></td>
<td>CVP</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

45 AO = 45 min of aortic occlusion; 30 EQ = 30 min equilibration; R = min of reperfusion.

Results

General Observations
There were no significant differences between the values for all parameters measured in the two sham groups. Consequently, they were combined for statistical purposes and are called the sham group. There were no differences in hemodynamics among the four other groups (table 1).

The comparisons of the effects of anesthetics and aortic occlusion–reperfusion on the other measured parameters are as follows.

Effect of Anesthesia and Xanthine Oxidase Inactivation on Pulmonary Alveolar–Capillary Membrane Permeability after Aortic Occlusion–Reperfusion
Desflurane anesthesia was associated with significantly greater bronchoalveolar lavage protein concentration and LDH activity after aortic occlusion–reperfusion when compared with fentanyl plus droperidol anesthesia \((P < 0.05; \text{fig. 1})\). Both aortic occlusion groups fed a standard diet had significantly greater bronchoalveolar lavage protein concentrations compared with the sham group \((P < 0.05; \text{fig. 1 [top]})\). However, only the aortic occlusion group administered desflurane anesthesia had significantly greater release of LDH activity into the bronchoalveolar lavage compared with the sham group \((P < 0.05; \text{fig. 1 [bottom]})\). Xanthine oxidase inactivation abrogated desflurane-dependent increases in bronchoalveolar lavage protein concentration and LDH activity after aortic occlusion–reperfusion \((P < 0.05, \text{fig. 1})\). There were no significant differences in lung tissue wet-to-dry weight ratios. The wet-to-dry weight ratios are as follows: sham \((5.54 ± 0.20)\); fentanyl plus droperidol, aortic occlusion \((5.62 ± 0.30)\); desflurane, aortic occlusion \((5.79 ± 0.63)\); and desflurane, sodium tungstate, aortic occlusion \((5.43 ± 0.38)\).

Effect of Anesthesia and Xanthine Oxidase Inactivation on Lung Tissue Xanthine Oxidase Activity and Ascorbic Acid Concentration after Aortic Occlusion–Reperfusion
Neither aortic occlusion–reperfusion nor the administered anesthetic significantly increased lung tissue xanthine oxidase activity (fig. 2, top). However, sodium tungstate administration significantly \((P < 0.05)\) decreased lung tissue xanthine oxidase activity by ~70–90% compared with the sham or desflurane-anesthetized aortic occlusion groups (fig. 2 [top]). Both aortic occlusion groups anesthetized with desflurane (standard and sodium tungstate diets) had significantly less \((~17\% , P < 0.05)\) lung tissue ascorbic acid content compared with the sham group (fig. 2 [bottom]). The aortic occlusion group anesthetized with fentanyl plus droperidol had lung tissue ascorbic acid content that was not different from that of either the sham or aortic occlusion groups anesthetized with desflurane (fig. 2 [bottom]).
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occlusion group fed a standard diet. Circulating xanthine oxidase activity significantly ($P < 0.05$) increased in both aortic occlusion groups fed a standard diet compared with the sham group, with no significant anesthetic-associated differences. Sodium tungstate administration resulted in a significant ($P < 0.05$) decrease in circulating xanthine oxidase activity after aortic occlusion–reperfusion, compared with the desflurane-anesthetized aortic occlusion group (~90% xanthine oxidase activity inactivation). Finally, aortic occlusion–reperfusion group, with desflurane anesthesia associated with significantly ($P < 0.05$) greater LDH activity release than fentanyl plus droperidol anesthesia after aortic occlusion–reperfusion (table 2). Sodium tungstate pretreatment resulted in a significant ($P < 0.05$) attenuation of LDH release compared with the desflurane aortic

Fig. 1. Effect of anesthesia and xanthine oxidase inactivation on pulmonary alveolar–capillary membrane injury after aortic occlusion–reperfusion. Groups are as follows: sham group (white bar); fentanyl plus droperidol anesthesia, aortic occlusion (hashed bar); desflurane anesthesia, aortic occlusion (black bar); and desflurane anesthesia, aortic occlusion, sodium tungstate diet (gray bar). Both aortic occlusion groups fed a standard diet had significantly greater bronchoalveolar lavage protein content compared with the sham group ($P < 0.05$). Desflurane anesthesia was associated with significantly greater bronchoalveolar lavage protein content and LDH activity after aortic occlusion–reperfusion when compared with fentanyl plus droperidol anesthesia ($P < 0.05$). However, only the aortic occlusion group given desflurane anesthesia had significantly greater release of LDH activity into the bronchoalveolar lavage compared with the sham group ($P < 0.05$). Xanthine oxidase inactivation abrogated desflurane-associated increases in bronchoalveolar lavage protein content and LDH activity after aortic occlusion–reperfusion ($P < 0.05$). Lavage = bronchoalveolar lavage. Values are means ± SD.

Fig. 2. Effect of anesthesia and xanthine oxidase inactivation on lung tissue xanthine oxidase activity and ascorbic acid content after aortic occlusion–reperfusion. The groups are as described in the legend to figure 1. Neither aortic occlusion–reperfusion nor the anesthetic administered significantly increased lung tissue xanthine oxidase activity. However, sodium tungstate administration significantly decreased lung tissue xanthine oxidase activity compared with the sham ($P < 0.05$) or desflurane-anesthetized aortic occlusion groups ($P < 0.05$). Both aortic occlusion groups anesthetized with desflurane (standard and sodium tungstate diets) had significantly lower ($P < 0.05$) lung tissue ascorbic acid content compared with the sham group. The aortic occlusion group anesthetized with fentanyl plus droperidol had lung tissue ascorbic acid content that was not different from either the sham or aortic occlusion groups anesthetized with desflurane. Values are means ± SD.

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**Table 2. Plasma Lactate Dehydrogenase Activity, Xanthine Oxidase Activity, and Protein Concentration**

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH (U·L⁻¹)</th>
<th>Protein (g·L⁻¹)</th>
<th>XO (mU·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>72 ± 28</td>
<td>34.8 ± 3.3</td>
<td>18 ± 17</td>
</tr>
<tr>
<td>Aortic occlusion + fentanyl + droperidol</td>
<td>1,238 ± 1,114*</td>
<td>23.7 ± 5.3*</td>
<td>291 ± 267*</td>
</tr>
<tr>
<td>Aortic occlusion + desflurane</td>
<td>2,040 ± 1,174*†</td>
<td>21.6 ± 6.0*</td>
<td>367 ± 224*</td>
</tr>
<tr>
<td>Aortic occlusion + desflurane + sodium tungstate</td>
<td>1,033 ± 815*‡</td>
<td>20.9 ± 3.7*</td>
<td>31 ± 54‡</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
LDH = lactate dehydrogenase; XO = xanthine oxidase.
* Aortic occlusion-reperfusion groups versus sham group, P < 0.05.
† Aortic occlusion-reperfusion group anesthetized with desflurane versus those anesthetized with fentanyl + droperidol, P < 0.05.
‡ Aortic occlusion-reperfusion group anesthetized with desflurane, pretreated with sodium tungstate versus aortic occlusion-reperfusion group anesthetized with desflurane, P < 0.05.

Perfusion resulted in significantly decreased plasma protein concentrations compared with the values of the sham group. However, there were no significant effects on plasma protein concentration due to the administered anesthetic or inactivation of endogenous xanthine oxidase activity.

*Influence of Circulating Xanthine Oxidase Activity on Alveolar–Capillary Membrane Permeability*

An increase in circulating xanthine oxidase activity at 180 min of reperfusion was significantly associated with increased bronchoalveolar lavage protein concentration (R = 0.41, P < 0.001) and LDH activity (R = 0.47, P < 0.001). A decrease in plasma protein concentration was significantly associated with an increase in bronchoalveolar lavage protein concentration (R = −0.33, P = 0.015). An increase in plasma LDH activity was significantly associated with an increase in bronchoalveolar lavage LDH activity (R = 0.68, P < 0.001). Finally, an increase in bronchoalveolar lavage protein concentration was significantly associated with an increase in bronchoalveolar lavage LDH activity (R = 0.62, P < 0.001).

*Effect of Anesthesia and Xanthine Oxidase Inactivation on Hemodynamics and Resuscitation Requirements*

Animals anesthetized with fentanyl plus droperidol required about 50% more lactated Ringer’s solution and twice as much phenylephrine to maintain hemodynamics after aortic occlusion-reperfusion compared with rabbits given desflurane (table 3). Administration of sodium tungstate to desflurane-anesthetized rabbits decreased phenylephrine requirements by about 50% after aortic occlusion-reperfusion compared with those fed a standard diet. There were no differences in sodium bicarbonate requirements after aortic occlusion-reperfusion among the groups.

*Effects of Anesthetics on Xanthine Oxidase Activity In Vitro*

Neither desflurane nor fentanyl plus droperidol significantly affected xanthine oxidase activity in vitro (table 4).

*Effect of Anesthesia and Xanthine Oxidase Inactivation on Partial Oxygen Pressure after Aortic Occlusion-Reperfusion*

There was no significant change in PaO₂ over time among the experimental groups. The PaO₂ values (measured in millimeters of mercury) for each group at 30 min of equilibration and 180 min of reperfusion, respectively, are as follows: sham-operated group (161 ± 13, 171 ± 21), aortic occlusion group given fentanyl plus droperidol (141 ± 26, 149 ± 27), aortic occlusion group given desflurane (164 ± 26, 157 ± 36), and aortic occlusion group given desflurane and sodium tungstate (155 ± 18, 170 ± 28).

**Discussion**

The current study provides the first in vitro evidence that desflurane (a halogenated anesthetic) increases pulmonary alveolar–capillary membrane permeability mediated by xanthine oxidase (a source of oxidant stress). Previously, in vitro investigations showed that halothane and isoflurane increased oxidant-mediated rat pulmonary artery endothelial cell injury by exposure to...
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Table 3. Resuscitation Requirements for Aortic Occlusion Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lactated Ringer’s Solution (ml·kg⁻¹·3 h⁻¹)</th>
<th>Phenylephrine (mg·kg⁻¹·3 h⁻¹)</th>
<th>Sodium Bicarbonate (mEq·kg⁻¹·3 h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl + droperidol</td>
<td>335 ± 100*</td>
<td>16.4 ± 10.6†</td>
<td>19.7 ± 6.1</td>
</tr>
<tr>
<td>Desflurane</td>
<td>212 ± 50</td>
<td>8.5 ± 4.5</td>
<td>20.5 ± 4.5</td>
</tr>
<tr>
<td>Desflurane + sodium tungstate</td>
<td>223 ± 38</td>
<td>4.6 ± 1.2‡</td>
<td>23.0 ± 4.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
Fentanyl + droperidol versus desflurane, *P < 0.001; †P < 0.05.
Desflurane versus desflurane + sodium tungstate, ‡P < 0.01.

activated neutrophils and exogenously administered H₂O₂. The concept that halogenated anesthetics can play a role in the tissue injury associated with oxidants was also supported by the ex vivo observation that halothane ventilation increased injury in isolated-perfused rabbit lungs exposed to tert-butyl-hydroperoxide. Mechanisms invoked to explain enhancement of oxidant-mediated cell or organ injury included possibilities such as halothane-mediated intracellular calcium mobilization or antioxidant depletion via a reduction of glutathione stores. In contrast to these findings, it was found that both isoflurane and halothane decreased H₂O₂-mediated human aortic endothelial cell injury in vitro, with decreased cytotoxicity associated with decreased calcium influx after exposure to halothane and isoflurane. However, neither halothane nor isoflurane protected human pulmonary endothelial cells from a similar oxidant stress. Similarly, both halothane and isoflurane decreased injury in a mouse model of multiple-organ dysfunction elicited by neutrophil activation via intraperitoneal injection of zymosan. Correspondingly, neutrophil migration and inflammatory cell free radical generation were inhibited by administration of halothane. However, neither droperidol nor fentanyl interfered with neutrophil function. These mostly in vitro and ex vivo data suggest that the interactions of halogenated anesthetics with oxidants are complex, sometimes contradictory, and poorly understood.

The precise mechanism by which desflurane increases xanthine oxidase-mediated pulmonary alveolar-capillary membrane permeability is not yet defined. In the present study, the lung was exposed to similar circulating xanthine oxidase activities after aortic occlusion-reperfusion with either desflurane or fentanyl plus droperidol anesthesia. Analogously, there was no significant increase in lung tissue xanthine oxidase activity due to the anesthetic administered. In vitro, no remarkable influence of desflurane or fentanyl plus droperidol on xanthine oxidase activity was observed. Although desflurane anesthesia was not associated with significantly increased circulating or tissue xanthine oxidase activity after aortic occlusion-reperfusion, desflurane-anesthetized rabbits had lower lung tissue ascorbic acid content after aortic occlusion-reperfusion compared with sham-operated animals. Interestingly, xanthine oxidase inactivation did not prevent the desflurane-associated decrease in lung tissue ascorbic acid content. Ascorbic acid is a water-soluble, primarily cytoplasmic antioxidant that scavenges nearly every oxidant that arises in biological systems, including O₂⁻, H₂O₂, ·OH, peroxyl radicals, and peroxyxynitrite. However, ascorbic acid may not be readily accessible to all xanthine oxidase-derived oxidants, depending on where the circulating enzyme has partitioned (e.g., the endothelial surface). Desflurane is reported to be minimally metabolized, so it is unlikely that lung antioxidant defenses were decreased because of the metabolism of desflurane in the lung. Thus desflurane may affect antioxidant defenses through an unknown direct mechanism or by causing the elaboration of other mediators having pro-oxidant properties.

Table 4. Effects of Anesthetics on Xanthine Oxidase Activity In Vitro

<table>
<thead>
<tr>
<th>Condition</th>
<th>Xanthine Oxidase (mU·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine oxidase</td>
<td>4.26 ± 0.14</td>
</tr>
<tr>
<td>Xanthine oxidase, desflurane</td>
<td>4.05 ± 0.15</td>
</tr>
<tr>
<td>Xanthine oxidase, fentanyl + droperidol</td>
<td>4.04 ± 0.20</td>
</tr>
</tbody>
</table>

Values are mean ± SD; six experiments (n = 6) per condition.

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LDH activity were used as markers of pulmonary alveolar-capillary membrane permeability and injury, as they have been used previously in human 33,35 and animal 36,40,41 studies, and have been found to correlate with histologic assessments of injury. 42,43 In a parallel study, 41 one of the authors (V.G.N.) found that lung injury graded histologically correlated significantly with bronchoalveolar lavage protein content (R = 0.51, P < 0.001) and LDH activity (R = 0.55, P < 0.001) in the rabbit model of aortic occlusion-reperfusion. Increases in bronchoalveolar lavage protein concentration and LDH activity may reflect regional injury to the lung for several reasons. Bronchoalveolar lavage protein accumulation was likely due to increased permeability of the pulmonary alveolar-capillary membrane in our model, because there was a negative correlation between plasma concentration of protein and lavage protein concentration. Furthermore, all three aortic occlusion groups had a similar plasma protein concentration but significantly different bronchoalveolar lavage protein concentrations. The decrease in plasma protein concentration in the aortic occlusion groups, compared with the sham-operated group, may be due in part to fluid administration or increased capillary permeability, especially in the vasculature exposed to ischemia-reperfusion (e.g., liver, intestine). Increases in bronchoalveolar lavage LDH activity correlated significantly with increases in bronchoalveolar lavage protein concentration (R = 0.62, P < 0.001) and increases in plasma LDH activity (R = 0.67, P < 0.001). Although it is possible that the release of LDH activity into the bronchoalveolar lavage may reflect an increase in permeability, it may also reflect local cell lysis in the lung for the following reasons. First, the lung tissue wet-to dry weight ratios were not significantly different among the groups and, if the lung was nonselectively permeable to a protein the size of LDH (140,000 daltons), we would expect a significant increase in lung edema. Second, although there is an approximately 14-fold difference in plasma LDH activity between the sham-operated group and the aortic occlusion group administered desflurane and sodium tungstate, there is no difference between the bronchoalveolar lavage LDH activity of the two groups. Finally, pulmonary alveolar-bronchiole epithelial cell lysis has been noted histologically after aortic occlusion-reperfusion in rabbits. 44

The resuscitative measures used to maintain hemodynamics did not significantly affect alveolar-capillary membrane permeability. There was no difference in the amount of sodium bicarbonate administered to the three aortic occlusion groups. Furthermore, the fentanyl plus droperidol-anesthetized group required significantly greater amounts of lactated Ringer’s solution and phenylephrine to maintain hemodynamics after aortic occlusion-reperfusion compared with the desflurane-anesthetized group fed a standard diet. Nevertheless, the group anesthetized with fentanyl plus droperidol had significantly less pulmonary alveolar-capillary membrane permeability after aortic occlusion-reperfusion compared with the desflurane-anesthetized group fed a standard diet. The differences in resuscitative measures after aortic occlusion-reperfusion observed between the two anesthetics administered may reflect their sympatholytic (fentanyl plus droperidol) and sympathomimetic (desflurane) properties. Overall, the anesthetic administered and xanthine oxidase inactivation were the primary determinants of the severity of pulmonary alveolar-capillary membrane permeability after aortic occlusion-reperfusion in the current study.

In conclusion, desflurane anesthesia was associated with increased pulmonary alveolar-capillary membrane permeability in a clinically relevant rabbit model of descending thoracic aorta occlusion-reperfusion compared with fentanyl plus droperidol anesthesia. Although not statistically different, the aortic occlusion group given desflurane was the only group to have a decrease in PaO₂ and the highest lung wet-to-dry weight ratio. We suspect that the lack of statistical significance in PaO₂ and lung wet-to-dry ratio may reflect a type 2 error (insufficient animals per group). The desflurane-associated increase in pulmonary alveolar-capillary membrane permeability observed after aortic occlusion-reperfusion occurred in the presence of similar circulating and tissue xanthine oxidase activities compared with those noted after fentanyl plus droperidol anesthesia. Inactivation of circulating and tissue xanthine oxidase activity with sodium tungstate abrogated desflurane-associated pulmonary alveolar-capillary membrane injury after aortic occlusion-reperfusion. Of interest, desflurane anesthesia was associated with increased oxidant stress, as indicated by decreased lung ascorbic acid content after aortic occlusion-reperfusion. These data raise the possibility that one of the mechanisms by which halogenated anesthetics may increase oxidant-mediated pulmonary alveolar-capillary membrane injury involves an anesthetic-mediated decrease in tissue antioxidant defenses. Future laboratory and clinical investigations are warranted to determine if desflurane and other halogenated anesthetics (e.g., sevoflurane) increase or decrease pulmonary injury during

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References


