Halothane and Xanthine Oxidase Increase Hepatocellular Enzyme Release and Circulating Lactate after Ischemia—Reperfusion in Rabbits

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Background: Multiple-organ injury often occurs after aortic occlusion—reperfusion. Oxidants derived from xanthine oxidase have been implicated as a source of injury after aortic occlusion—reperfusion. Halogenated anesthetics modify oxidant-mediated injury. The current study determined if halothane modifies hepatocellular enzyme release (e.g., alanine aminotransferase) and circulating lactate after aortic occlusion—reperfusion.

Methods: Rabbits were randomly assigned to one of four groups that underwent 40 min of thoracic aortic occlusion and 2 h of reperfusion: Two groups were given either halothane or fentanyl plus droperidol anesthesia and two groups were given either anesthetic and sodium tungstate (xanthine oxidase inactivator). Each of the four groups was then matched with a similarly treated group that did not undergo aortic occlusion.

Results: Halothane anesthesia was associated with significantly (P < 0.05) increased release of alanine aminotransferase (34 ± 9 U/l at baseline and 539 ± 370 U/l at 120 min of reperfusion; mean ± SD) and increased plasma lactate concentrations (2.8 ± 2.0 mm at baseline and 12.1 ± 9.7 mm at 120 min of reperfusion) after aortic occlusion—reperfusion compared with fentanyl plus droperidol anesthesia (alanine aminotransferase, 33 ± 12 U/l and 148 ± 109 U/l; lactate, 3.4 ± 2.0 mm and 3.8 ± 1.2 mm at baseline and 120 min of reperfusion, respectively). Inactivation of xanthine oxidase significantly decreased the release of hepatocellular enzymes (P < 0.05) and decreased circulating lactate in animals anesthetized with halothane after aortic occlusion—reperfusion.

Conclusions: Halothane increased hepatocellular enzyme release and circulating lactate after aortic occlusion—reperfusion compared with fentanyl plus droperidol anesthesia. Xanthine oxidase activity inactivation also decreased hepatocellular enzyme activity release during reperfusion. These findings justify further investigations to determine if halogenated anesthetics modify tissue injury in clinical settings involving oxidant stress.

(Key words: Anesthetics, volatile; halothane. Liver injury; ischemia. Enzymes; xanthine oxidase; alanine aminotransferase; aspartate aminotransferase. Hypoperfusion: lactate.)

MULTIPLE-ORGAN injury occurs after trauma and major vascular surgery and affects thousands of patients annually.1-3 Hepatic ischemia commonly occurs in these settings secondary to overt shock or as a consequence of surgical interventions (e.g., aortic cross-clamping). Although age and concurrent organ dysfunction increase the risk of organ failure after hepatic ischemia,1,2 the release of xanthine oxidase into the circulation plays a significant role.4-9 Of interest, the liver and intestine contain the highest tissue activity of the enzyme and its precursor, xanthine dehydrogenase.9 In this article, both forms of the enzyme are called “xanthine oxidase.” In addition to oxidant stress, patients often share another common experience in the perioperative period—anaesthesia. Halogenated anesthetic agents are often administered in these settings.10-12 Although it is generally accepted that oxidants play a role in tissue injury, the role of anesthetic agents remains poorly understood.

Halothane is metabolized in the liver, which may result in the production of toxic intermediates13,14 and carbon-centered radical species.15 The hepatic oxygen uptake/supply relation is also decreased in vivo by halo-
HALOTANE AND XANTHINE OXIDASE INCREASE ISCHEMIC INJURY

genated anesthetic agents. However, halogenated anesthetic agents decrease hepatocyte oxygen consumption in vitro and hepatic oxygen uptake in vivo. Consequently, halogenated anesthetic agents could minimize hepatic ischemia–reperfusion injury by reducing basal metabolism or could increase injury by decreasing organ oxygen supply, increasing the formation of toxic radical species and increasing the release of circulating mediators of oxidant stress such as xanthine oxidase. Halothane has also been shown to increase or decrease endothelial injury in vitro and increase oxidant-mediated lung injury ex vivo. The role played by halogenated anesthetic agents in the evolution of hepatic ischemia–reperfusion injury is controversial and requires further investigation.

We performed this study to determine if halothane modifies hepatic injury as measured by hepatocellular enzyme release after descending thoracic aorta occlusion–reperfusion as compared with an intravenous anesthetic (fentanyl plus droperidol). We hypothesized that halothane-associated hepatocellular enzyme release could be modulated by inactivating xanthine oxidase activity. We used the release of hepatocellular enzymes as an estimate of the extent of hepatic injury because their release has been correlated with histologic injury in other models of liver ischemia. Finally, we hypothesized that halothane and xanthine oxidase activity could influence systemic perfusion during reperfusion as determined by changes in circulating lactate.

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, Washington, D.C., US Government Printing Office).

Experimental Groups

Male, New Zealand, white rabbits (Myrtle's Rabbits, Thompson Station, TN) weighing 2–3 kg were randomly administered a standard diet (n = 36) or a molybdenum-deficient, sodium tungstate-enriched diet (n = 36; 0.07% w/w sodium tungstate; Purina Mills, St. Louis, MO) for 10 days before the experiments. Previous research has shown that administration of sodium tungstate results in a significant reduction in tissue and plasma xanthine oxidase activity in rabbits. The animals were fasted for 14–16 h before the experiments but were allowed free access to water.

All rabbits were anesthetized with 10 mg/kg intravenous ketamine (Parke-Davis, Morris Plains, NJ) given via a marginal ear vein. Animals were randomly assigned to one of the following maintenance anesthetic regimens: (1) 100 mg·kg⁻¹·h⁻¹ intravenous fentanyl (Elkins-Sinn, Cherry Hill, NJ) and 5 mg·kg⁻¹·h⁻¹ droperidol (American Reagent Laboratories, Shirley, NY) given via a marginal ear vein, or (2) inhaled 1% halothane (Abbott Laboratories, North Chicago, IL) carried in oxygen. Halothane administration (inspired concentration) was monitored with an airway gas monitor (model 254; Datex, Helsinki, Finland) that was calibrated daily. After anesthesia was induced, incision sites were infiltrated subcutaneously with 1% lidocaine for additional analgesia.

Rabbits were also randomly assigned to undergo sham operation (Sham) or aortic occlusion–reperfusion. Consequently, there were a total of eight groups (n = 9 per group): (1) sham, fentanyl plus droperidol, standard diet; (2) aortic occlusion–reperfusion, fentanyl plus droperidol, standard diet; (3) sham, halothane, standard diet; (4) aortic occlusion–reperfusion, halothane, standard diet; (5) sham, fentanyl plus droperidol, tungstate diet; (6) aortic occlusion–reperfusion, fentanyl plus droperidol, tungstate diet; (7) sham, halothane, tungstate diet; and (8) aortic occlusion–reperfusion, halothane, tungstate diet.

Surgical Protocol

Arterial pressure was monitored by placing a 22-gauge central ear artery catheter and a right femoral arterial catheter. After tracheostomy, mechanical ventilation (fraction of inspired gas in oxygen = 0.99–1.0) was performed with the partial pressure of carbon dioxide in arterial blood maintained at 32–45 Torr (4.2–6.0 kPa). Pancuronium bromide (Elkins-Sinn) at a dose of 0.1 mg·kg⁻¹·h⁻¹ was administered intravenously. 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 Ringer's solution at 20 ml·kg⁻¹·h⁻¹, and esophageal temperatures were maintained at 38–39°C with a heating pad. We
used Ringer’s solution (nonlactated) to avoid confounding lactate measurements with the administration of exogenous lactate. 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 thoracic aorta occlusion beginning with ligation of the femoral artery. The aortic occlusion – reperfusion 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 1-2 cm above the diaphragm as confirmed by postmortem examination. Thoracic aorta occlusion was achieved by inflating the catheter balloon with saline. Subdiaphragmatic ischemia was confirmed by a femoral arterial pressure of 0–10 Torr. After 40 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 ear arterial line. Postocclusion shock was treated according to the algorithms described in Resuscitation Protocol. Arterial blood samples were obtained at baseline conditions (preanesthetic) and at 120 min of reperfusion. The blood was centrifuged and the plasma assayed for release of the hepatocellular enzyme (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and lactate dehydrogenase [LDH]) activity as described in Biochemical Analysis. Whole blood lactate samples were similarly collected and determined as cited in Biochemical Analysis. Lactate concentration served as a measure of the adequacy of systemic perfusion. After 2 h of reperfusion, the rabbits were killed with a 65 mg/kg overdose of pentobarbital.

**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.  

**Ringer’s Administration.** At the beginning of reperfusion, a bolus (20 ml/kg) was administered over 2 min, and the infusion rate was adjusted to maintain central venous pressure at the preclosure value ± 1 mmHg.

**Phenylephrine Administration.** Phenylephrine (Elkins-Sinn) administration began at reperfusion and was adjusted as follows: If the central venous pressure was preclosure value ± 1 mmHg, and the mean arterial blood pressure was < 85% of the preclamp value, phenylephrine was administered.

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

**Biochemical Analysis**

**Alanine Aminotransferase, Aspartate Aminotransferase, and Lactate Dehydrogenase Activity.** Fresh plasma samples were allowed to come to room temperature before spectrophotometric assay. The ALT, AST, and LDH activity was determined using a modification of the procedure of Henry et al.  

**Lactate.** Whole blood was deproteinized by the addition of ice cold 8% perchloric acid (1:2 v/v) followed by vortexing. The samples were centrifuged at 15,000g for 15 min, and the supernatant stored at -85°C before assay. Lactate concentration was determined according to a modification of the method of Marbach and Weil.  

**Protein Assay.** Plasma samples were assayed for total protein concentration by a modification of the method of Smith et al. The assay involves the reaction of protein with Cu²⁺ in an alkaline medium (yielding Cu⁴⁺) with a highly sensitive and selective detection reagent for Cu⁴⁺, bicinchoninic acid. The plasma values obtained were used to normalize hepatocellular enzyme activity to account for the effects of resuscitation and blood sampling. The method used was a modification of a formula used by Taggart et al. to account for acute dilutional changes in plasma protein concentration. The formula used is as follows: Enzyme activity = measured current enzyme activity × baseline protein concentration/current protein concentration.

**Xanthine Oxidase.** Plasma samples were prepared and processed as previously described. Briefly, samples were subjected to size exclusion chromatography with a 25-gauge column (Pharmacia, Piscataway, NJ) to remove low molecular weight inhibitors of xanthine oxidase. 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 xanthine oxidase activity. Xanthine oxidase activity was determined from the rate of production of uric acid in the presence of xanthine (75 mm) and nicotinamide adenine dinucleotide (NAD+, 0.5 mm). One unit of activity (U) is defined as 1 μmol/min urate formed at 37°C and pH 7.4. Allopurinol (100 mm), an inhibitor of xanthine oxidase, was used in parallel samples to confirm that urate formation was specifically due to xanthine oxidase activity. After 60 min of incubation at 37°C,
HALOTHANE AND XANTHINE OXIDASE INCREASE ISCHEMIC INJURY

Table 1. Hepatocellular Enzyme Activities and Circulating Lactate Concentrations for Sham-operated Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U·L⁻¹)</th>
<th>AST (U·L⁻¹)</th>
<th>LDH (U·L⁻¹)</th>
<th>Lactate (mmol·L⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>F + D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>28 ± 9</td>
<td>13 ± 2</td>
<td>45 ± 5</td>
<td>2.8 ± 2.5</td>
</tr>
<tr>
<td>120 min</td>
<td>30 ± 8</td>
<td>17 ± 7</td>
<td>85 ± 32</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td>F + D, T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>31 ± 15</td>
<td>13 ± 8</td>
<td>48 ± 16</td>
<td>4.0 ± 2.2</td>
</tr>
<tr>
<td>120 min</td>
<td>35 ± 15</td>
<td>18 ± 9</td>
<td>98 ± 22</td>
<td>3.0 ± 1.7</td>
</tr>
<tr>
<td>Halothane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>27 ± 7</td>
<td>13 ± 7</td>
<td>50 ± 9</td>
<td>2.9 ± 2.0</td>
</tr>
<tr>
<td>120 min</td>
<td>29 ± 9</td>
<td>18 ± 8</td>
<td>74 ± 31</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>Halothane, T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>24 ± 8</td>
<td>9 ± 3</td>
<td>34 ± 11</td>
<td>2.3 ± 2.2</td>
</tr>
<tr>
<td>120 min</td>
<td>30 ± 12</td>
<td>15 ± 5</td>
<td>75 ± 14</td>
<td>1.2 ± 0.7</td>
</tr>
</tbody>
</table>

Values are mean ± SD. 
ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; F + D = fentanyl + droperidol anesthesia; T = sodium tungstate diet.

the reaction was terminated by deproteinization with acetonitrile. The uric acid content of deproteinized plasma samples was determined using a high-power liquid chromatography-based electrochemical technique.²⁸

Additional data concerning the effect of ischemia-reperfusion and xanthine oxidase on myocardial injury were collected in the fentanyl plus droperidol anesthetized animals and are published elsewhere.²⁹

Statistical Analysis
All variables are expressed as mean ± SD. The hypothesis that halothane and xanthine oxidase inactivation modifies hepatocellular injury after aortic occlusion-reperfusion was tested as follows. The change from baseline to 120 min of reperfusion was determined for all variables and groups. The analyses for each variable (ALT, AST, and LDH activity; lactate concentration; xanthine oxidase activity) were conducted with one-way analyses of variance (ANOVA). Given that any four outcomes (ALT, AST or LDH activity, lactate concentration) may indicate injury, a Bonferroni correction for the alpha level to account for multiple comparisons was made (P < 0.0125 for one-way ANOVA). The analyses testing for anesthetic and xanthine oxidase mediated differences in resuscitation requirements (phenylephrine, Ringer’s solution, and sodium bicarbonate) were conducted with ANOVA. The Student-Newman-Keuls test was used for post hoc comparisons, with an alpha error < 0.05 considered significant.

Results
General Observations
There were no statistical differences between the sham-operated animals with regard to any of the measured injury parameters (table 1). There were no clinically significant anesthetic-mediated hemodynamic differences noted between either the sham-operated or aortic occlusion-reperfusion groups (table 2). The results of the differences in measures of hepatocellular injury between the aortic occlusion-reperfusion groups are as follows.

Effect of Anesthesia and Xanthine Oxidase Inactivation on Alanine and Aspartate Aminotransferases and Lactate Dehydrogenase Activity Release and Circulating Lactate Concentration after Aortic Occlusion-Reperfusion
There were significant (P < 0.001 by one-way ANOVA) differences in ALT, AST, and LDH activity among the eight experimental groups (fig. 1). Halothane anesthesia was associated with significantly increased (P <

Table 2. Mean Arterial and Central Venous Pressures for All Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mmHg)</th>
<th>CVP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min EQ</td>
<td>120 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham, F + D</td>
<td>76 ± 6</td>
<td>75 ± 14</td>
</tr>
<tr>
<td>Sham, F + D, T</td>
<td>76 ± 10</td>
<td>77 ± 17</td>
</tr>
<tr>
<td>Sham, halothane</td>
<td>83 ± 18</td>
<td>65 ± 11</td>
</tr>
<tr>
<td>Sham, halothane, T</td>
<td>82 ± 9</td>
<td>63 ± 13</td>
</tr>
<tr>
<td>AO, F + D</td>
<td>82 ± 7</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>AO, F + D, T</td>
<td>70 ± 5</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>AO, halothane</td>
<td>77 ± 15</td>
<td>77 ± 9</td>
</tr>
<tr>
<td>AO, halothane, T</td>
<td>85 ± 14</td>
<td>82 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SD. 
F + D = fentanyl + droperidol anesthesia; T = sodium tungstate diet; 30 min EQ = 30 min of equilibration; 120 min = 120 min of observation. For brevity, only 30 min EQ and 120 min data are shown.

Fig. 1. Effect of anesthesia and xanthine oxidase inactivation on alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activity release and circulating lactate after aortic occlusion–reperfusion. Groups were fentanyl plus droperidol anesthesia, aortic occlusion–reperfusion (black circles, F&D); halothane anesthesia, aortic occlusion–reperfusion (black triangles, halothane); fentanyl plus droperidol anesthesia, aortic occlusion–reperfusion, sodium tungstate diet (open circles, F&D, T); and halothane anesthesia, aortic occlusion–reperfusion, sodium tungstate diet (open triangles, halothane, T). Halothane anesthesia significantly increased circulating ALT, AST, and LDH activity and lactate concentration compared with fentanyl plus droperidol anesthesia after aortic occlusion–reperfusion ($P < 0.05$). Inactivation of xanthine oxidase activity with sodium tungstate significantly decreased circulating ALT, AST, and LDH activity and lactate concentration activity after aortic occlusion–reperfusion in halothane-anesthetized animals ($P < 0.05$). Only the occlusion–reperfusion groups are depicted in the figure. Values for the matched sham-operated groups are shown in table 1. Values are mean ± SD.

0.05) circulating ALT, AST, and LDH activity compared with fentanyl plus droperidol anesthesia after aortic occlusion–reperfusion. Inactivation of xanthine oxidase activity resulted in a significant decrease ($P < 0.05$) in ALT, AST, and LDH release after aortic occlusion–reperfusion in halothane-anesthetized animals. Although the increase in circulating hepatocellular enzyme release was decreased in fentanyl plus droperidol–anesthetized rabbits administered sodium tungstate, compared with animals fed a standard diet, this difference was not significant ($P > 0.05$).

There were significant ($P < 0.001$ by one-way ANOVA) differences in circulating lactate concentration among the eight experimental groups. Halothane anesthesia resulted in significantly greater ($P < 0.05$) circulating lactate concentrations after aortic occlusion–reperfusion when compared with fentanyl plus droperidol anesthesia. Fentanyl plus droperidol anesthesia was noted to have no significant affect on circulating lactate concentration after aortic occlusion–reperfusion compared with sham-operated animals. The inactivation of xanthine oxidase activity significantly ($P < 0.05$) de-
HALOTHANE AND XANTHINE OXIDASE INCREASE ISCHEMIC INJURY

Table 3. Resuscitation Requirements for Aortic Occlusion–Reperfusion Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Phenylephrine (mg·kg⁻¹·2 h⁻¹)</th>
<th>Ringer’s (ml·kg⁻¹·2 h⁻¹)</th>
<th>Sodium Bicarbonate (mEq·kg⁻¹·2 h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F + D</td>
<td>7.1 ± 6.1</td>
<td>322 ± 81*</td>
<td>16.7 ± 2.4*</td>
</tr>
<tr>
<td>F + D, T</td>
<td>9.1 ± 9.4</td>
<td>301 ± 77†</td>
<td>13.9 ± 1.7</td>
</tr>
<tr>
<td>Halothane</td>
<td>6.1 ± 5.6</td>
<td>212 ± 65</td>
<td>22.0 ± 5.7†</td>
</tr>
<tr>
<td>Halothane, T</td>
<td>4.6 ± 2.9</td>
<td>228 ± 53</td>
<td>16.4 ± 1.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
F + D = fentanyl + droperidol anesthesia; T = sodium tungstate diet.
*P < 0.05, F + D versus halothane.
†P < 0.05, F + D, T versus halothane, T.
‡P < 0.05, halothane versus halothane, T.

increased circulating lactate concentrations (by about three times) in animals anesthetized with halothane after aortic occlusion–reperfusion.

Effect of Anesthesia and Xanthine Oxidase Inactivation on Circulating Xanthine Oxidase Activity after Aortic Occlusion–Reperfusion

Complete circulating xanthine oxidase activity data for the fentanyl plus droperidol groups were reported in another article. In summary, circulating xanthine oxidase activity in rabbits administered a standard diet at baseline conditions averaged 51 ± 80 mU/l. Pretreatment with sodium tungstate reduced this baseline value to an average of 7 ± 11 mU/l. In animals fed a standard diet, reperfusion was associated with a significant (P < 0.05) increase in xanthine oxidase activity. With fentanyl plus droperidol, xanthine oxidase activity increased to 565 ± 440 mU/l. This was significantly (P < 0.05) reduced to 93 ± 81 in animals pretreated with sodium tungstate. In contrast, xanthine oxidase activity increased to 1,046 ± 551 mU/l in halothane-anesthetized animals (P < 0.05 vs. fentanyl plus droperidol-anesthetized animals), with values significantly reduced to 231 ± 236 after sodium tungstate pretreatment.

Effect of Anesthesia and Xanthine Oxidase Inactivation on Resuscitation Requirements

There was no significant difference in phenylephrine requirements among the four aortic occlusion–reperfusion groups (table 3). There were significant (P < 0.005 by one-way ANOVA) differences in Ringer’s requirements among the four aortic occlusion–reperfusion groups. Animals anesthetized with fentanyl plus droperidol required significantly (P < 0.05) more Ringer’s solution than did those anesthetized with halothane, with no differences due to xanthine oxidase inactiva-

Discussion

Our study shows that hepatic ischemia–reperfusion injury (as shown by ALT, AST, and LDH release) is in-
increased by halothane anesthesia and attenuated by inactivation of xanthine oxidase. In contrast to hepatocellular enzyme activity release, circulating lactate concentration remained increased after aortic occlusion-reperfusion in only the halothane-anesthetized animals, with xanthine oxidase inactivation significantly attenuating the lactate acidemia. The differential effects of anesthetics and xanthine oxidase activity inactivation on circulating lactate concentration provide some insight into the possible mechanisms of hepatocellular injury after aortic occlusion-reperfusion.

Perturbation of the hepatic oxygen supply-and-demand relation is a possible mechanism that could explain the halothane-associated hepatocellular enzyme activity release observed after aortic occlusion-reperfusion. In support of this concept, studies involving halothane-anesthetized guinea pigs showed a marked decrease in hepatic blood flow associated with reduced oxygen saturation in blood from the portal vein.

However, other studies involving humans and dogs, did not document significant changes in the hepatic oxygen supply-and-demand relation during halothane anesthesia. These conflicting data are likely due to a species variation in the response to halothane anesthesia. In the setting of hepatic ischemia-reperfusion, halothane-anesthetized pigs had an increased hepatic oxygen supply:uptake ratio. However, hepatic lactate uptake was only 20-40% of the baseline value during reperfusion in these animals. These data support the possibility that the regional hepatic hemodynamic/metabolic effects are due to ‘‘stunning’’ or overt tissue loss after ischemia. Our study supports this scenario, with halothane anesthesia associated with increased release of hepatocellular enzyme activity and persistently increased circulating lactate concentrations after aortic occlusion-reperfusion. If sufficiently injured, the liver may not be able to metabolize the increased circulating lactate load associated with aortic occlusion-reperfusion, resulting in increased circulating lactate concentrations. However, the persistently increased circulating lactate concentrations observed during halothane anesthesia after aortic occlusion-reperfusion may also represent prolonged regional ischemia in other reperfused organs (e.g., the intestines) compared with fentanyl plus droperidol anesthesia. Halothane-associated hepatocellular injury may be due to persistent hepatic hypoperfusion or tissue injury after aortic occlusion-reperfusion.

In addition to modifying oxygen supply-demand relations, halothane modulates oxidant-mediated tissue injury in vitro and ex vivo. A critical in vitro investigation by Shayevitz et al. showed that halothane and isoﬂurane increased rat pulmonary artery endothelial cell injury mediated by activated neutrophils and direct application of hydrogen peroxide. The concept that halogenated anesthetics can play a role in 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/organ injury included possibilities such as halothane-mediated intracellular calcium mobilization or antioxidant depletion via reduction of glutathione stores. In contrast to these findings, Johnson et al. found that isoﬂurane and halothane decreased H$_2$O$_2$-mediated human aortic endothelial cell injury in vitro. Decreased cell death was 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. Halogenated anesthetics are markedly protective against injury in the setting of myocardial ischemia-reperfusion.
HALOTHANE AND XANTHINE OXIDASE INCREASE ISCHEMIC INJURY

larly. Shayevisz et al. found that 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 free radical generation were inhibited by administration of halothane. However, neither droperidol nor fentanyl interfere with neutrophil function. These data as a whole suggest that the interactions of halogenated anesthetics with oxidants are highly complex and poorly understood. There are likely organ-specific (liver vs. heart), oxidant-specific, oxidant generator-specific (neutrophil vs. xanthine oxidase), and species-specific differences in the tissue injury observed during oxidant stress and administration of halogenated anesthetics.

The effects of halogenated anesthetics on ischemia-reperfusion injury are like a double-edged sword. The severity of tissue ischemia-reperfusion injury depends on several factors that include oxidant stress, antioxidant status, the oxygen supply-demand ratio, and the tissue or cells studied. If halothane decreases the metabolic rate in a tissue (e.g., the heart), then we would expect a decrease in ischemia-reperfusion injury. If neutrophils are central to the specific injury scenario, then a halothane-mediated decrease in neutrophil function may be beneficial. However, the liver and intestine have high tissue xanthine oxidase activities. Consequently, hepatoenteric ischemia-reperfusion results in significant circulating xanthine oxidase-mediated oxidant stress. This oxidant stress, coupled with halothane anesthesia, may result in increased injury due to decreased hepatic blood flow and concomitant oxidant-mediated injury. Hepatic metabolism of halothane also consumes reduced nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide phosphate, which may decrease hepatocyte antioxidant defenses (e.g., glutathione) dependent on reducing equivalents. Consequently, the liver may be exposed to increased circulating oxidant stress while cellular antioxidant defenses and the oxygen supply-demand ratio decrease. Persistent halothane-mediated hepatic hypoperfusion could result in further xanthine oxidase release, creating a vicious cycle of halothane- and oxidant-mediated tissue injury. The halothane-mediated hepatoenteric ischemia-reperfusion injury observed in the current study supports this scenario.

Hepatoenteric ischemia-reperfusion results in the release of xanthine oxidase activity into the circulation. Xanthine oxidase typically exists in an innocuous form (xanthine dehydrogenase) in nonischemic tissues, with purine catabolism occurring concomitantly with the reduction of nicotinamide adenine dinucleotide. When tissues are exposed to metabolic stress, such as hypoxia or ischemia, xanthine dehydrogenase converts to the oxidase form. Xanthine oxidase, in the presence of adequate purine substrate and molecular oxygen, generates the oxidants superoxide anion, hydrogen peroxide and hydroxyl radical. Although conversion of xanthine dehydrogenase to xanthine oxidase occurs in tissues during a period of hours by irreversible proteolysis, rapid conversion of xanthine dehydrogenase to xanthine oxidase occurs within seconds by reversible oxidation of sulphhydrals once released into plasma. The release of xanthine oxidase alone is not sufficient to produce tissue injury under normal conditions because xanthine oxidase activity is limited by low purine substrate concentrations (1-3 μM) in the systemic circulation. However, plasma concentrations of the xanthine oxidase substrates, hypoxanthine and xanthine, have been documented to increase after ischemia-reperfusion in humans during reconstructive aortic surgery. Xanthine oxidase and high concentrations of purine substrates could be released into the circulation after hepatoenteric ischemia-reperfusion and consequently produce systemic injury.

The difference in hepatocellular enzyme release caused by aortic occlusion-reperfusion was significant only in the halothane-anesthetized groups. However, increases in xanthine oxidase activity release after aortic occlusion-reperfusion was significant within both anesthetics. Xanthine oxidase colocalizes with other hepatocellular and intestinal enzymes. We consequently suspect that the lack of statistical significance in hepatocellular enzyme release due to aortic occlusion-reperfusion in the fentanyl plus droperidol-anesthetized groups may reflect a type II error (insufficient animals per group).

With regard to resuscitation requirements, it is notable that the requirements for phenylephrine were not different among the aortic occlusion-reperfusion groups and did not correlate with increased injury. This of particular clinical interest given the commonly held clinical impression that increased α-agonists may increase ischemic visceral injury via constriction of the visceral vascular beds. The differences in requirements for Ringer’s solution may simply reflect the different vasoactive properties of the two anesthetics (with fentanyl plus droperidol being sympathetic). On the other hand, halothane-anesthetized animals may have a decreased “reflow” to the microcirculation compared
with animals anesthetized with fentanyl plus droperidol, resulting in decreased fluid requirements. Halothane anesthesia was associated with significantly increased sodium bicarbonate requirements during reperfusion that was attenuated by xanthine oxidase inactivation. Notably, increases in circulating xanthine oxidase correlated significantly with increased sodium bicarbonate requirements during reperfusion. There are anesthetic-specific and xanthine oxidase-mediated effects on sodium bicarbonate requirements during reperfusion that may reflect ongoing systemic hypoperfusion, but phenylephrine administration is not significantly influenced by either the administered anesthetic or xanthine oxidase inactivation. The patterns of resuscitation requirements likely reflect the effects of halothane and xanthine oxidase activity on ischemia–reperfusion injury, not the converse situation.

In conclusion, halothane anesthesia increased the release of hepatocellular enzyme activity in a clinically relevant rabbit model of descending thoracic aorta occlusion–reperfusion compared with fentanyl plus droperidol anesthesia. Xanthine oxidase activity inactivation was also associated with decreased hepatocellular enzyme activity release after aortic occlusion–reperfusion. The lactate concentration data support the possibility that halothane contributes to hepatocellular injury by causing hypoperfusion (regional or systemic) or by other as yet identified mechanisms. Similarly, the lactate data support the possibility that in addition to oxidant-mediated cytotoxicity, xanthine oxidase activity may mediate regional tissue ischemia that exacerbates hepatocellular injury. These data serve as a rational basis for further investigations of the precise mechanism(s) responsible for halothane-mediated injury in the setting of oxidant stress. Given the frequency of the administration of halogenated anesthetics, further human investigation of the effect of halogenated anesthetics on multi-organ injury in settings such as trauma and major vascular surgery is warranted.

References


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