High tidal volume ventilation induces lung injury after hepatic ischemia-reperfusion

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Ota S, Nakamura K, Yazawa T, Kawaguchi Y, Baba Y, Kitaoka R, Morimura N, Goto T, Yamada Y, Kurahashi K. High tidal volume ventilation induces lung injury after hepatic ischemia-reperfusion. Am J Physiol Lung Cell Mol Physiol 292: L625–L631, 2007. First published October 20, 2006; doi:10.1152/ajplung.00151.2006.—Ischemia-reperfusion not only damages the affected organ but also leads to remote organ injuries. Hepatic inflow interruption usually occurs during hepatic surgery. To investigate the influence of liver ischemia-reperfusion on lung injury and to determine the contribution of tidal volume settings on liver ischemia-reperfusion-induced lung injury, we studied anesthetized and mechanically ventilated rats in which the hepatic inflow was transiently interrupted twice for 15 min. Two tidal volumes, 6 ml/kg as a low tidal volume (IR-LT) and 24 ml/kg as a high tidal volume (IR-HT), were assessed after liver ischemia-reperfusion, as well as after a sham operation, 6 ml/kg (NC-LT) and 24 ml/kg (NC-HT). Both the IR-HT and IR-LT groups had a gradual decline in the systemic blood pressure and a significant increase in plasma TNF-α concentrations. Of the four groups, only the IR-HT group developed lung injury, as assessed by an increase in the lung wet-to-dry weight ratio, the presence of significant histopathological changes, such as perivascular edema and intravascular leukocyte aggregation, and an increase in the bronchoalveolar lavage fluid TNF-α concentration. Furthermore, only in the IR-HT group was airway pressure increased significantly during the 6-h reperfusion period. These findings suggest that liver ischemia-reperfusion caused systemic inflammation and that lung injury is triggered when high tidal volume ventilation follows liver ischemia-reperfusion.

Acute lung injury; ventilator-induced lung injury; lung protective strategy; cytokines

ISCHEMIA-REPERFUSION NOT ONLY damages the affected organ itself but can also cause systemic inflammation (32, 34). Such systemic inflammation may lead to remote organ injury and morbidity (20). Nevertheless, transient interruption of hepatic inflow is sometimes necessary during partial hepatectomy (10, 14). The lung is one of the most susceptible organs to systemic inflammation-related injury, secondary to liver ischemia-reperfusion (7, 16, 36), intestinal ischemia-reperfusion (6, 37), and renal ischemia-reperfusion (11, 17).

Acute lung injury (ALI), or acute respiratory distress syndrome (ARDS), is an important cause of mortality in critically ill patients. The mortality rate from ALI or ARDS is ~40–50% (4, 31). In some cases, ALI or ARDS can be caused by extrapulmonary factors, such as systemic inflammation (5, 25) and sepsis (30, 35). ALI/ARDS patients ventilated with low tidal volume ventilation have a lower mortality rate than those ventilated with high tidal volume ventilation (3, 4). There are in vivo and ex vivo studies that have analyzed the effect of tidal volume on ventilator-induced lung injury (VILI) following LPS administration (2, 28, 33) and cecal ligation and perforation (23); both of these induce severe systemic inflammation. As far as we know, there are no published papers that have explored the effect of different tidal volumes on VILI in connection with liver ischemia-reperfusion.

To evaluate the effects of ventilation on lung injury induced by systemic inflammation following organ ischemia-reperfusion, we used a rat liver ischemia-reperfusion model with two ventilation settings (high tidal volume, 24 ml/kg, and low tidal volume, 6 ml/kg) and compared the pulmonary function and histopathological changes.

MATERIALS AND METHODS

Animal preparation. All protocols for the animal experiments were approved by the Animal Research Committee of Yokohama City University. Specific pathogen-free male Sprague-Dawley rats, weighing 320–400 g (9 to 11 wk; Japan SLC, Shizuoka, Japan), were used for all animal experiments. Anesthesia was induced by injecting 25 mg of pentobarbital sodium into the peritoneal cavity. A tracheotomy was performed, and a 14-gauge plastic cannula (SR-OT1451C; Terumo, Tokyo, Japan) was inserted into the trachea and served as a tracheal port. Mechanical ventilation was maintained using a constant volume pump (SN-480-7; Shinano, Tokyo, Japan) with an inspired oxygen concentration of 1.0. Tidal volume (VT) delivered by the ventilator was calibrated volumetrically by collecting expired gas. In brief, the ventilator circuit was connected to a test lung that would generate an intracircuit pressure of −20 cmH2O while expiratory gas was collected from the expiration port. Tidal volume was set to 6 ml/kg from the beginning of ventilation to the end of the second hepatic inflow obstruction; PEEP was not applied during liver ischemia-reperfusion. The ventilation frequency was adjusted to maintain the arterial carbon dioxide tension between 35 and 50 Torr. The right carotid artery was catheterized with a 24-gauge plastic cannula (SR-OT2419C, Terumo) to measure the blood pressure (BP) and sample arterial blood. A right femoral vein was catheterized with a 24-gauge plastic cannula (SR-OT2419C, Terumo) to allow continuous infusion of hydroxyethyl starch (HES). HES was infused at a rate of 10 ml/kg·h−1 for the first hour and then at a rate of 5 ml/kg·h−1.

BP and airway pressure were monitored continuously using a hemodynamic monitor (Life Scope 12; Nihon Kohden, Tokyo, Japan). A rectal probe (ME-PDK061, Terumo) was used to continuously monitor body temperature (CTM-303, Terumo). The body temperature was maintained between 36°C and 38°C by placing the animals on a warming device. Blood (1 ml) was sampled every hour for blood gas analysis and TNF-α measurement. Blood gas and acid-base
analyses were performed using a critical care analyzer (OPTI3; AVL Scientific, Roswell, GA). The volume of withdrawn blood was replaced by the same volume of Ringer lactate solution (R/L) administered intravenously. In addition, 1 ml of R/L was given intra-arterially at an interval of 10, 5, 2, or 1 min when the systolic BP decreased to 100, 90, 80, or 70 mmHg, respectively. Six hours after reperfusion, the rats were deeply anesthetized and euthanized.

Liver ischemia-reperfusion. All procedures were performed using sterile technique. The hepatic artery and portal vein were isolated and encircled with 3.0 silk; the hepatic inflow was interrupted by placing a vascular clip on the hepatic artery and portal vein. Complete hepatic inflow obstruction could be confirmed immediately by the liver becoming pale in color. Ischemia was induced twice for 15 min with an interval of 5 min between the two interruptions. Rats in the sham operation groups received the same procedure, including hepatic artery and portal vein isolation, except that vascular clamping with a clip was not done.

Experimental groups. Fifty-two rats were used to evaluate lung injury and the systemic responses to liver ischemia-reperfusion. After the second liver ischemia-reperfusion episode, the rats were randomized to receive either high VT (VT 24 ml/kg, PEEP 0 cmH2O: IR-HT) or low VT + low PEEP (VT 6 ml/kg, PEEP 3 cmH2O: IR-LT). This time point was defined as time 0. Control animals had a laparotomy and were randomized to receive either high VT (VT 24 ml/kg, PEEP 0 cmH2O) or low VT + low PEEP (VT 6 ml/kg, PEEP 3 cmH2O) ventilation starting at time 0, which occurred 35 min after the isolation of the hepatic artery and portal vein.

Bronchoalveolar lavage. A set of eight rats in each group had bronchoalveolar lavage (BAL) fluid collected 6 h after reperfusion. BAL fluid was collected twice with 1.5 ml of PBS containing 0.1% EDTA per lavage. The fluid that was obtained was centrifuged at 3,400 g at 4°C for 20 min to obtain the supernatant and stored at −80°C until use.

Measurement of liver and lung injury. Liver injury was assessed by measuring plasma transaminases, aspartate transaminase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH) using a clinical chemistry automated analyzer (Modular Analytics; Hitachi, Tokyo, Japan). Lung injury was assessed in two different ways: 1) the lungs of one subset of rats from each group were used to determine the lung wet-to-dry weight ratio (W/D), which indicates lung edema. The W/D of the lungs was calculated in the established manner (29, 35). Each lung was harvested 6 h after reperfusion and individually homogenized, placed in a preweighed aluminum pan, and dried to a constant weight in an oven at 80°C for 3 days; 2) the lungs of another subset of rats from each group were used for histopathological examination. These lungs were fixed with 4% formalin in PBS overnight and then processed for paraffin-embedded sections. Four-micrometer-thick lung tissue sections were stained with hematoxylin and eosin for routine histological examination. The histopathology was assessed by a pathologist who was blinded to the protocol and experimental groups. The pathologist assessed and scored the degree of perivascular edema, intravascular leukocyte aggregation, and leukocyte infiltration in the alveolar space.

Leukocyte count. The leukocyte counts in the systemic circulation and in the BAL fluid were quantified microscopically. Arterial blood (25 μl) was obtained 6 h after reperfusion and was added to 475 μl of 2% acetic acid containing 0.01% Gentian Violet. BAL fluid (25 μl) was added to 25 μl of 2% acetic acid containing 0.01% Gentian Violet. Cell counts were multiplied by the dilution factor to obtain the number of leukocytes in the blood or BAL fluid.

Assay for TNF-α in the BAL fluid and blood. A biological TNF-α assay was done using mouse sarcoma cells, WEHI-13VAR (CRL2148, American Type Culture Collection, Manassas, VA), as previously reported (15, 18). The TNF-α activity of each sample was calculated by comparing absorbance to that of a standard curve made from dilutions of rat TNF-α (PharMingen, San Diego, CA) between 1.2 pg/ml and 1,250 pg/ml. The lower limit of detection of this assay is 1.2 pg/ml.

Statistical analysis. The mean values of the measurements that were taken only once during the protocol were compared using unpaired t-tests. Measurements that were made more than once per animal were compared using repeated measures ANOVA. Pairwise comparisons were made by one-factor ANOVA followed by Scheffe’s post hoc analysis. Values are reported as means ± SE.

RESULTS

Hemodynamics, acid-base status, plasma TNF-α, and liver injury. BP and base excess (BE) were stable in the NC-LT and NC-HT groups (Fig. 1, A and B). Hepatic inflow interruption caused rapid and progressive decrease in BP in these groups; the BP returned to baseline values just after reperfusion but then gradually decreased during the 6-h reperfusion period. The mean BP in the IR-LT and IR-HT groups was significantly lower than in the NC-LT and the NC-HT groups 6 h after reperfusion; the mean BP in the IR-HT group was significantly lower than in the IR-LT group (Fig. 1A). In the IR-LT and IR-HT groups, the rats required extra fluid to maintain their BP during liver ischemia and reperfusion periods; this was not necessary in the NC-LT and NC-HT groups (Table 1). In the IR-LT group, the BE deteriorated markedly during hepatic inflow interruption and then gradually rose to the normal range during the reperfusion period, although it was still significantly lower than in the NC-LT and the NC-HT groups 6 h after reperfusion. The BE in the IR-HT group deteriorated during liver ischemia, and severe acidosis continued during the reperfusion period (Fig. 1B). In the sham operation groups, plasma TNF-α concentration transiently and mildly increased between 30 and 60 min after surgery and then returned to the baseline value. Between 30 and 60 min after reperfusion, plasma TNF-α concentrations in the IR-LT and the IR-HT groups were ~100 times more than in the NC-LT and NC-HT groups (Fig. 1C). The plasma concentrations of AST, ALT, and LDH after 6 h of reperfusion were elevated slightly in the NC-LT and NC-HT groups; in the IR-LT and IR-HT groups, the concentrations were much higher than in the sham operation groups (Fig. 1D).

There were no significant differences in AST and ALT between tidal volumes within groups that had the same procedure.

Peak airway pressure and Pao2. Thirty minutes after randomization, peak airway pressure (Paw) in the NC-LT and IR-LT groups increased in proportion to the applied PEEP, 3 cmH2O, and did not change for 6 h (Fig. 2A). At 30 min after randomization, the peak Paw in the NC-HT and IR-HT groups was about twice as that noted in the NC-LT and IR-LT groups. The peak Paw in the NC-HT group did not change for 6 h, whereas the peak Paw of the IR-HT group became gradually elevated and was significantly higher than in any of the other groups at 360 min (Fig. 2A).

Oxygenation did not change in the NC-LT, NC-HT, and IR-LT groups during the 6-h reperfusion period, whereas in the IR-HT group, oxygenation deteriorated during this time period (Fig. 2B).

Lung W/D ratio and BAL fluid analysis. There was a trend toward a higher W/D ratio in the NC-HT group compared with the NC-LT group, but it was not statistically significant (Fig. 3A). The W/D ratio in the IR-LT group was similar to that in
the NC-LT group. However, the IR-HT group had a significantly higher W/D ratio than the NC-LT and IR-LT groups.

The recovered BAL fluid volume was similar in all experimental groups (Table 1). The BAL fluid TNF-α concentrations were similar in the NC-LT, NC-HT, and IR-LT groups, but the TNF-α concentration in the IR-HT group was significantly higher than in any of the other groups (Fig. 3B).

Histopathological study. On histopathology, an almost normal lung structure was noted in the NC-LT group (Fig. 4A). The alveolar spaces were enlarged in both the NC-HT and IR-HT groups (Fig. 4, B and D). Adhesion of leukocytes on the vascular walls was frequently seen in the IR-LT and IR-HT groups (Fig. 4, C and D). Obstruction of small vessels due to leukocyte aggregation with occasional microthrombus formation was detected in the IR-HT group (Fig. 4D) but was not observed in the other groups. In all groups, leukocyte infiltration into the alveolar space was minimal.

These pathological findings are scored and summarized in Table 2.

**Table 1. Resuscitation fluid, collected BAL fluid, and leukocyte counts in the blood and BAL fluid of the four experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Resuscitation fluid volume, ml</th>
<th>Recovered BAL fluid volume, ml</th>
<th>Leukocytes in blood (μl)</th>
<th>Leukocytes in BAL fluid (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC-LT</td>
<td>0</td>
<td>2.02±0.11</td>
<td>2.443±334</td>
<td>1.176±112</td>
</tr>
<tr>
<td>NC-HT</td>
<td>0</td>
<td>2.11±0.13</td>
<td>3.539±427</td>
<td>1.318±220</td>
</tr>
<tr>
<td>IR-LT</td>
<td>12.8±2.6*†</td>
<td>1.85±0.12</td>
<td>2.090±360</td>
<td>2.177±246</td>
</tr>
<tr>
<td>IR-HT</td>
<td>29.1±3.4*†‡</td>
<td>2.23±0.06</td>
<td>1.708±396†</td>
<td>960±156‡</td>
</tr>
</tbody>
</table>

Resuscitation fluid, collected bronchoalveolar lavage (BAL) fluid, and the number of leukocytes are expressed as means ± SE. Note that the resuscitation fluid includes the volume of the fluid administered to maintain blood pressure but does not include the volume of the infusion used to replace the volume of blood lost due to sampling. NC-LT, no clamp of the hepatic artery and portal vein and low tidal volume (VT) ventilation (VT 6 ml/kg, PEEP 3 cmH2O). NC-HT, no clamp of the hepatic artery and portal vein and high VT ventilation (VT 24 ml/kg, PEEP 0 cmH2O). IR-LT, hepatic ischemia-reperfusion and low VT ventilation (VT 6 ml/kg, PEEP 3 cmH2O). IR-HT, hepatic ischemia-reperfusion and high VT ventilation (VT 24 ml/kg, PEEP 0 cmH2O). *P < 0.05 vs. NC-LT group. †P < 0.05 vs. NC-HT group. ‡P < 0.05 vs. IR-LT group. §P < 0.05 vs. IR-HT group.

**DISCUSSION**

We demonstrated the effects of different tidal volumes on lung injury in the liver ischemia-reperfusion model. Previous reports dealing with various systemic inflammatory states have shown that mechanical ventilation with large VT causes a deterioration in lung function (19, 28). The transient hepatic inflow interruption that is often done to reduce blood loss in hepatic surgery (10, 14) causes liver ischemia-reperfusion injury and results in systemic inflammation (34, 36). As far as we know, there have been no published reports that have directly demonstrated the effects of different tidal volumes on lung injury under liver ischemia-reperfusion. In the present study, we mimicked the hepatic blood-flow interruption that occurs during hepatic surgery and found that the animals...
developed lung injury after liver ischemia-reperfusion only when the lungs were ventilated with a high tidal volume.

There are several reports that have dealt solely with the effect of different tidal volumes on lung injury without the presence of any other confounding factors. Intermittent positive-pressure ventilation with high inflation pressure has been found to induce pulmonary edema (12, 13). Our observation that the NC-HT group had a tendency to higher W/D than the NC-LT group is in agreement with these previous studies. Recently, the cytokine profile in the air spaces with VILI has been studied. Tremblay et al. (33) showed that, in isolated nonperfused rat lungs ventilated with a large V_T, significant amounts of cytokines, including TNF-α, were released into the air spaces. Using the same ex vivo model, Ricard et al. (27) found that the TNF-α level was negligible, regardless of the ventilatory conditions. They also tested the same V_T in vivo and found that the animals’ BAL fluid contained no TNF-α (27). In the present study, the rats ventilated with a high V_T following a sham operation had low BAL fluid TNF-α levels, and these levels were similar to those seen in rats ventilated with low V_T following a sham operation. Lung histology and the cell counts in the BAL fluid showed that there was no increase in the number of infiltrating cells in the alveolar spaces in either of the sham operation groups. These findings suggest that, in our experimental model, high V_T ventilation per se does not directly cause lung injury to the level that would promote the elevation of TNF-α levels in the air spaces or the occurrence of histopathological changes.

The slight elevations of AST, ALT, and LDH levels that were seen in the NC-LT and NC-HT groups were considered to have occurred as a result of the sham operation. The extremely high levels of enzymes in the IR-LT and IR-HT groups that were noted were the result of hepatic cell damage caused by hepatic inflow interruption. Tidal volume had minimal effects on AST and ALT levels.

In both the IR-LT and IR-HT groups, the TNF-α concentration levels found in the circulation increased and peaked

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Fig. 2. Peak airway pressure (A) and change in PaO_2 (B). A: peak airway pressure was continuously measured. BL, baseline value taken just before the interruption of hepatic inflow. Reperfusion was started at time 0. B: change of PaO_2 from the value obtained 30 min after reperfusion as an indicator of oxygenation. Means ± SE; *P < 0.05 vs. NC-LT group, ¶P < 0.05 vs. NC-HT group, §P < 0.05 vs. IR-LT group. #P < 0.05 vs. IR-HT group 30 min after reperfusion.

Fig. 3. Assessment of lung injury. A: lung wet-to-dry weight ratio. B: concentration of TNF-α in bronchoalveolar lavage (BAL) fluid. BAL was performed 6 h after reperfusion. *P < 0.05 vs. NC-LT group. ¶P < 0.05 vs. NC-HT group. §P < 0.05 vs. IR-LT group.
30–60 min after reperfusion. Although a transient increase of the plasma TNF-α concentration was also observed in both the NC-LT and NC-HT groups, the peak plasma TNF-α concentrations in both the IR-LT and the IR-HT groups were much higher, almost 100 times more than in the NC-LT and NC-HT groups. Given that macrophages are known to be able to produce a high amount of TNF-α and that the liver contains the largest fixed population of macrophages (Kupffer cells) in the body (7, 9), the liver macrophages are considered to be the source of circulating TNF-α. Of note, although the exact mechanism of TNF-α elevation in BAL fluid and its relationship to systemic TNF-α are unknown, only the IR-HT group had a high concentration of BAL fluid TNF-α. These findings suggest that two conditions must be fulfilled in this experimental model so that TNF-α is increased in the alveolar space: the presence of liver ischemia-reperfusion and ventilation with high VT. The results of previous in vitro and ex vivo studies support this hypothesis. Pugin et al. (26) analyzed cytokine secretion from isolated alveolar macrophages subjected to a pressure-stretching strain resembling that of conventional mechanical ventilation. Neither the pressure-stretching strain alone, nor the incubation of cells with lipopolysaccharide alone, but only the combination of these two factors, produced significant amounts of TNF-α (26). Ricard et al. (27) showed significant TNF-α production in the BAL fluid of ventilated isolated, nonperfused rat lungs when the rats were given lipopolysaccharide before

Table 2. Scoring of the histopathological changes

<table>
<thead>
<tr>
<th></th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC-LT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NC-HT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IR-LT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IR-HT (3)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Obstruction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC-LT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NC-HT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IR-LT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IR-HT (3)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Infiltration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC-LT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NC-HT (3)</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>IR-LT (3)</td>
<td>3</td>
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<tr>
<td>IR-HT (3)</td>
<td>3</td>
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</table>

Perivascular edema (Edema), small vessel obstruction due to leukocyte aggregation (Obstruction), and leukocyte infiltration into the alveolar space (Infiltration) were assessed by a pathologist in a blinded manner. These observations were scored using three grades: Grade 1, no or mild change; Grade 2, moderate change; or Grade 3, marked change.
lung removal. Given these observations, one can conclude that liver ischemia-reperfusion primes pulmonary cells to produce TNF-α when the lungs are ventilated with high VT. Thus a useful ventilatory strategy to reduce lung injury would be to reduce VT when hepatic inflow interruption has occurred.

In the IR-HT group, in addition to the TNF-α increase seen in the BAL fluid, leukocyte adhesion to vascular walls, small vessel obstruction, and perivascular fluid accumulation were noted on histopathology. Previous studies have shown that liver ischemia-reperfusion induces polymorphonuclear neutrophil (PMN) recruitment in the lung (21). Neutrophil recruitment at sites of inflammation depends on the expressions of P-selectin and ICAM-1 on the endothelium (1, 24). The expressions of both of these molecules are regulated by several cytokines, including TNF-α. Thus the histopathological findings in the IR-HT group could be considered to be at least partly the result of the increase in circulating TNF-α. However, this does not offer a complete explanation since these findings were not observed in the IR-LT group that had a high circulating TNF-α concentration. The high TNF-α concentration in the alveolar space of the IR-HT group might be responsible for the adherence of the PMNs to the pulmonary vascular walls. Thus, further investigation of other mediators, such as adhesion molecules and chemokines, is warranted.

In the present study, the infiltration of PMNs into the air space was minimal in all groups. Colletti et al. (8, 9) showed intra-alveolar PMN infiltration 12 h after reperfusion that followed 90 min of lobar hepatic ischemia. In contrast, Matuschak et al. (22) showed a lung PMN influx 1 h after reperfusion that was not present 24 h after reperfusion. The reason for such diverse results is thought to be due to variations in the experimental design, including differences in: the duration of ischemia; the type of hepatic inflow obstruction (total or partial); respiration during the reperfusion period; the timing of the examinations after reperfusion; and the animal species that were studied.

We used 100% oxygen in all animals during the experiment since the animals might not have survived hepatic ischemia with hypotension or might have experienced hypoxemia during the 6-h reperfusion period and might have died. However, 100% oxygen itself might have to some extent augmented the degree of lung injury.

In conclusion, liver ischemia-reperfusion causes systemic inflammation, but not lung injury, when lungs are ventilated with a low tidal volume. Lung injury is triggered by high tidal volume ventilation after liver ischemia-reperfusion. Therefore, a careful ventilatory strategy is necessary to prevent ischemia-reperfusion-induced lung injury.

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