Lung Injury After Aortic Occlusion-Reperfusion in Rats: the Role of Gadolinium Chloride

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Lung Injury After Aortic Occlusion-Reperfusion in Rats: the Role of Gadolinium Chloride. Tohoku J. Exp. Med., 2004, 203 (4), 267-273 — Aortic ischemia-reperfusion (AIR) induced lung injury has already been documented. Kupffer cell blockage (KCB) with gadolinium chloride (GdCl₃) has also been shown to attenuate remote organ damage caused by ischemia reperfusion. The present study was designed to examine the effect of GdCl₃ in lung ischemia-reperfusion injury induced by aortic occlusion. Thirty-two rats were randomly allocated to four groups as follows: SHAM (Sham Laparotomy), SHAM+KCB, AIR, and AIR+KCB. An atraumatic microvascular clamp was placed across the infrarenal abdominal aorta just after its origin from the aorta for 30 minutes. The microvascular clamp on the infrarenal abdominal aorta was removed and reperfused for 60 minutes. GdCl₃ was given 24 hours prior to the experiment. Malondialdehyde (MDA) level and myeloperoxidase (MPO) activity were assayed in lung tissues. MDA level and MPO activity in the AIR group were significantly higher than those in the other groups. When compared to AIR group, KCB with GdCl₃ significantly decreased MDA level and MPO activity in the AIR+KCB group. These results suggest that GdCl₃ attenuates the lung injury caused by AIR. The effects of GdCl₃ on reduced lung damage may be mediated through significant decreases in both MDA level and MPO activity. ——— gadolinium chloride; ischemia-reperfusion; lung

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Aortic cross-clamping is usually performed in aortic surgery. Hemodynamic and metabolic changes are usually mild and transient in temporary aortic cross-clamping. When aortic occlusion is carried out, there has been obstruction to blood supply of lower extremities. Morbidity and mortality rate is related with the duration and degree of ischemia and mass of tissue involved. Remote organ damage could be developed if the ischemic injury is severe (Blaisdell 1989). Xanthine oxidase (XO) and its precursor were released from reperfusing liver and intestine (Terada et al. 1992; Weinbroum et al. 1995). Acute aortic occlusion with subsequent ischemia-reperfusion (IR) of the lower extremities is known to predispose to lung injury (Axon et al. 1998; Tassiopoulos et al. 1998; Sookhai et al. 2002). The release of XO after hepatenteric IR plays a major role in the evaluation of lung injury (Terada et al. 1992; Koike et al. 1993; Weinbroum et al. 1995; Nielsen et al. 1997).

Gadolinium chloride (GdCl₃), a rare earth lanthanide, has been used for abrogate macrophage migration and activation in vivo to delineate their role in disease (Tullis et al. 1996; Jankov et al. 2001). The Kupffer cells, the resident macrophage of the liver and lung, are the major component of mononuclear phagocytic system (MPS). Kupffer cells appear to contribute to the modulation of remote organ damage after intestinal IR (Towfight et al. 2000). In animals, GdCl₃ has been reported to be effective in preventing pulmonary injury induced by ozone inhalation and ovine lentivirus infection (Pendino et al. 1995; Singh and dela Concha-Bermejillo 1998). In contrast, the effect of GdCl₃ in humans has not yet studied in this respect. Since the liver is the largest source of resident macrophages in the body, it has been proposed that transhepatic passage of intestinal reperfusate has a stimulatory effect on these cytokine-producing cells (Tullis et al. 1996).

The effect of GdCl₃ on lung damage induced by the occlusion of the infrarenal abdominal aorta has not yet been investigated. Therefore, we studied the role of GdCl₃ in lung injury caused by occlusion of the infrarenal abdominal aorta IR.

**Material and Methods**

**Animal model**

Thirty-two Wistar-Albino rats in both sex and weighed between 200 to 250 g were used in the study. The animals were acquired from the university vivarium sources and were housed in individual cages in a temperature and light-dark cycle controlled environment with free access to food and water. Food, but not water, was withheld 12 hours prior to experiment. All animals received humane care, in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animals Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). Our protocol and experimental methods were approved by the Animal Care and Use Committee of the Laboratory Animal Service of the University of Suleyman Demirel.

**Technique of aortic ischemia-reperfusion**

Kupffer cell blockage was done by intravenous administration of GdCl₃ (Sigma Chemical Co., St. Louis, MO, USA) at a dose of 10 mg/kg 24 hours before the experiment (Tullis et al. 1996). The rats were anaesthetised with an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Istanbul, Turkey) and were placed under a heating lamp. The skin was aseptically prepared and a midline laparotomy was performed. Ten ml of warm normal saline was instilled into the peritoneal cavity to help maintain fluid balance. The abdominal aorta was exposed by gently deflecting the loops of intestine to the left with moist gauze swabs. An atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int., St. Paul, MN, USA) was then placed across the infrarenal abdominal aorta.
(IAA) for 30 minutes. The abdomen was then closed and the wound was covered with plastic wrap to minimise heat and fluid losses. The microvascular clamp on the IAA was removed and reperfused for 60 minutes. Aortic occlusion and reperfusion were confirmed by the loss and reappearance of satisfactory pulsation in the distal aorta. Hence, no-reflow phenomenon was excluded. All animals were killed under anaesthesia, lungs were carefully removed en bloc from the thorax. The specimens were harvested and stored at −78°C until biochemical assays. Time-matched, sham operated animals undergoing laparotomy and dissection of the IAA without occlusion served as controls for the experiment.

**Experimental design**

Thirty-two rats were randomly allocated to four groups as follows: SHAM (Sham Laparotomy), SHAM+KCB, AIR, and AIR+KCB.

**Biochemical procedure**

The frozen tissue samples of lung were weighed and homogenised (Ultra Turrax T25, Janke & Kunkel GmbH & Co., KG, Staufen, Germany) (1:10, w/v) in 100 mmol/liter phosphate buffer (pH 7.4) containing 0.05% sodium azide in an ice bath. The homogenate was sonicated (Bandelin Sonoplus UW 2070, Berlin, Germany) for 30 seconds and centrifuged (5000 g for 10 minutes). The supernatant was frozen at −78°C in aliquots until used for biochemical assays. The protein content of the supernatant was determined using the Lowry method (Lowry et al. 1951).

**Malondialdehyde assay**

Malondialdehyde (MDA) levels, an indicator of free radical generation which increase at the end of the reperfusion, were estimated by the double heating method of Draper and Hadley (1990). The principle of the method is the spectrophotometric measurement of the colour generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 ml of 100 g/liter trichloroacetic acid solution was added to 0.5 ml supernatant in each centrifuge tube and the tubes were placed in a boiling water bath for 15 minutes. After cooling in tap water, the tubes were centrifuged at 1000 g for 10 minutes and 2 ml of the supernatant was added to 1 ml of 6.7 g/liter TBA solution in a test tube and the tube was placed in a boiling water bath for 15 minutes. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Shimadzu, Kyoto) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA-TBA complex (absorbance coefficient \( \epsilon = 1.56 \times 10^5 \) cm M⁻¹) and is expressed as nanomoles per milligram of protein (nmol/mg protein).

**Myeloperoxidase assay**

Myeloperoxidase (MPO) activity, a sensitive index of tissue polymorphonuclear leukocytes (PMNL) sequestration, was determined in the rat lung by using the peroxidase-catalysed, \( \text{H}_2\text{O}_2 \)-dependent oxidantion of tetramethylbenzidine as a measure of enzymatic activity (Tullis et al. 1996). Lung specimens were weighed as an 1 g and placed in a 9 ml 50 mM potassium phosphate buffer of pH 6 with 0.5% hexadecyltrimethylammonium bromide (Sigma). The specimens were homogenised (Ultra-Turrax T25, Janke & Kunkel GmbH & Co. KG, Staufen, Germany) for 20 seconds in an ice bath. The homogenate was sonicated (Bandelin Sonopuls UW 2070, Berlin, Germany) for 30 seconds and centrifugated at 12 000 g for 15 minutes at 4 °C. The supernatant was assayed for MPO content spectrophotometrically by measuring the change in optical density at 460 nm over time. The assay buffer consisted of 50 mM potassium phosphate, pH 6.0 (50 ml), 0.83 ml \( \text{H}_2\text{O}_2 \) (0.3% solution; Sigma) and 8.34 mg o-dianisidine hydrochloride (Sigma). The supernatant was mixed 1 : 80 (supernatant: assay buffer). MPO units are expressed as unit/g lung tissue protein.
**Statistical analysis**

Data are presented as means ± s.d. error. Differences between groups were determined using one way ANOVA followed by a post hoc Tukey’s honestly significant difference test. The level of significance was set at \( p < 0.05 \). A computer program (SPSS 10.01, SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

**RESULTS**

MDA levels (nmol/mg protein) and MPO activity (unit/g lung tissue protein) in the lung of four groups were shown in Table 1. There were no significant differences in MDA levels and MPO activity among three groups (SHAM, SHAM+KCB and AIR+KCB). In contrast, MDA levels and MPO activity in the AIR group of lungs were significantly higher than that in these groups (SHAM, SHAM+KCB and AIR+KCB) \( (p<0.05) \). When compared to AIR group, Kupffer cell blockage with GdCl\(_3\) significantly decreased MDA levels and MPO activity in the AIR+KCB group \( (p<0.05) \).

**DISCUSSION**

Remote lung injury, as assessed by MDA level and MPO activity in lung, was observed after aortic IR. KCB significantly reduced MDA level and MPO activity in lung after AIR. Our data suggest that GdCl\(_3\) plays an important role in the AIR-induced lung damage through blocking Kupffer cell activity.

A decrease in the blood flow to an organ results in ischemic damage. But when blood flow is restored a more pronounced damage, so called reperfusion injury, occurs. The enhanced generation of oxygen radicals has been suggested in the development of IR injury (Schoenberg and Beger 1993).

Rats subjected to aortic IR had increased the extent of lipid peroxidation in lung (assessed by MDA level) and lung neutrophil retention (assessed by MPO activity). Levels of MDA, an indicator of free radical generation, increase at the end of reperfusion. In the present study, MDA levels were reduced in lung by GdCl\(_3\). Therefore, GdCl\(_3\) may reduce lung damage and free radical formation in AIR injury.

MPO activity is used as an indirect evidence of neutrophil infiltration in oxidant induced tissue injury, and it has been reported that MPO activity correlates well with the neutrophil count (Goldblum et al. 1985; Tullis et al. 1996). Although there is no evidence which supports that GdCl\(_3\) is able to suppress the function of neutrophil, there is a report showing that GdCl\(_3\) can suppress the accumulation of neutrophil and also alveolar macrophage (Sato et al. 2002).

The significance of this study would have been enhanced considerably by analysis of classical markers such as wet/dry lung weight ratio, the degree of extravascular lung water, and histopathological examination. Microscopic evaluation of lung tissue and PMN leukocyte counting were not performed in this study, because lung MPO activity reflects well the

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA</th>
<th>MPO</th>
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<tr>
<td>SHAM (n=8)</td>
<td>3.50±0.19</td>
<td>44.14±1.95</td>
</tr>
<tr>
<td>SHAM+KCB (n=8)</td>
<td>3.64±0.17</td>
<td>42.25±2.89</td>
</tr>
<tr>
<td>AIR (n=8)</td>
<td>5.36±0.36*</td>
<td>66.31±1.06*</td>
</tr>
<tr>
<td>AIR+KCB (n=8)</td>
<td>3.63±0.28</td>
<td>47.38±3.08</td>
</tr>
</tbody>
</table>

KCB, Kupffer Cell Blockage; AIR, Aortic Ischemia Reperfusion; MDA, Malondialdehyde (nmol/mg protein); MPO, Myeloperoxidase (unit/g lung tissue protein).

\(*p<0.05\) (compared to other groups)
neutrophil count in lung tissue. Wet/dry lung weight ratio is a reliable method for documenting lung edema, evaluation of PMN leukocyte sequestration rather than lung edema, but the present study focused on evaluation of PMN leukocyte sequestration rather than lung edema. Thus, microscopic neutrophil counting and wet/dry lung weight ratio were not performed and only lung MPO activity was measured. The present study demonstrates increased MPO activity in lung tissue following AIR. Furthermore, MDA level and MPO activity were reduced to control levels by GdCl₃.

Lipid based cell membranes are the primary target of these oxygen derived free radicals. When they react directly with polyunsaturated fatty acids, leading to lipid peroxidation within the cell membranes followed by disintegration of the cells and ultimately to cell death. End products of lipid peroxidation include MDA, other aldehydes, hydrocarbon gases and conjugated dienes (Savas et al. 2003). Besides their direct damage to the tissue, free radicals seem to trigger the accumulation of neutrophils in the tissue (McMillen et al. 1993). Free radicals stimulate monocytes to produce various cytokines such as tumour necrosis factor-α (TNF) and interleukin-1 (IL-1) (Hirose et al. 2000). These cytokines act on endothelial cells to promote the production of interleukines and are also responsible for respiratory burst of neutrophils and release of free radicals (Hirose et al. 2000). This cascade might explain the preventative role of GdCl₃ on IR injury. In the present study, GdCl₃ significantly decreased the levels of MDA, which is an end product of free radical formation and lipid peroxidation.

In fact, it has been clearly demonstrated that XO activity displays an important role both in the etiology of remote lung and heart injury in the animal model of hepatoenteric ischaemia-reperfusion (Terada et al. 1992; Koike et al. 1993; Weinbroum et al. 1995; Nielsen et al. 1997) and in the leukosequestration and acute lung injury which were significantly attenuated by inhibition or inactivation of XO (Axon et al. 1998).

In the present study, instead of XO activity, MDA level and MPO activity, as indicators of lung damage, were measured. We believed that the measurement of MDA level and MPO activity is sufficient to assess the lung damage. On the other hand, the mechanism responsible for lung injury was previously studied; and it was reported that the production of reactive oxygen species primarily involved in the induction of lung injury (Axon et al. 1998).

To the best of our knowledge, the present study provides the first in vivo evidence indicating that GdCl₃ decreases damage of lung after aortic occlusion-reperfusion. It has been previously shown that GdCl₃ significantly decreased lung cytokine levels, PMNL sequestration and free radical formation after intestinal IR (Savas et al. 2003). GdCl₃ abrogates macrophage accumulation by induction of apoptosis after phagocytosis, but has little effect on PMN leukocytes (Jankov et al. 2001). KCB by means of GdCl₃ administration reduced the total number of MPS cells contributing to the pulmonary damage, suggesting that MPS may play an important role in the development of acute lung injury after intestinal IR (Jankov et al. 2001).

Kupffer cells are the primary source of amplification of proinflammatory cytokine release following intestinal ischemia. These hepatic macrophages make up 90% of the fixed tissue macrophages and they are the major component of the MPS. It has been reported that Kupffer cells are responsible for the increased levels of TNF, IL-1, IL-6 in trauma, haemorrhagic shock and resuscitation. Decreasing the number or functional ability of Kupffer cells can lead to decreased levels of inflammatory cytokines as seen in the models of liver resection and sepsis (Towfigh et al. 2000; Savas et al. 2003).

Macrophages are differentiated from mononuclear phagocytes that may be residents in tissues for several months. Macrophages are essential for tissue remodelling wound healing and congregate during subacute or chronic inflammation. Although we did not determine the
total number of MPS cells, there have been many reports (Lazar 1973; Hardonk et al. 1992; Tullis et al. 1996; Jankov et al. 2001) indicating that GdCl₃ can reduce the total number or the function of MPS cells.

We also speculated that whether GdCl₃ could be given at one hour or just prior to aortic cross-clamping, rather than the 24 hours pre-treatment. This is relevant in the clinical setting of ruptured abdominal aortic aneurysm where there is a higher incidence of remote organ injury compared to elective aortic surgery, and would not allow for a major pre-treatment period. However, we aimed to eliminate Kupffer cells completely in this study. In order to achieve this goal, GdCl₃ should be given 24 hours prior to surgery (Tullis et al. 1996). Since there was no direct measurement of activity of Kupffer cells, the discussion of the blocking effect of GdCl₃ on the activity of Kupffer cells might be speculative. However, it has been previously well documented that administration of GdCl₃ at a dose of 10 mg/kg blocks the phagocytic activity and selectively eliminate the Kupffer cells from the periportal zone of the liver acinus after a single injection (Tullis et al. 1996). Also, the occlusion may be performed of the descendent aorta, not infrarenal abdominal aorta. Because, occlusion of the descendent aorta may be more related with clinical settings than infrarenal abdominal aorta.

In summary, the results of present study indicate that GdCl₃ has an important ability to prevent in lung injury due to AIR.

**CONCLUSION**

The present study provides the first-ever in vivo evidence showing that administration of GdCl₃ attenuates lung injury after aortic occlusion-reperfusion. We conclude that GdCl₃ significantly decreases MDA level and MPO activity in lung after AIR. One possible mechanism by which GdCl₃ protects against lung injury after aortic occlusion-reperfusion includes decreasing the number or functional ability of Kupffer cells leading decreased levels of inflammatory cytokines. It is evident that GdCl₃ reduces free radical formation in lung.

We suggest that GdCl₃ may reduce the total number of mononuclear phagocytic system cells, which play an important role in lung injury after AIR. The results of present study may improve our understanding in order to reduce mortality and morbidity due to lung injury during the setting of aortic occlusion-reperfusion by the administration of GdCl₃. In future, there may be a need for GdCl₃ pre-treatment in the aortic procedures. However, further experimental and clinical studies are needed in order to clarify the exact mechanism.

**Abbreviations**

(XO), Xanthine oxidase; (IR), Ischemia-Reperfusion; (GdCl₃), Gadolinium chloride; (MPS), Mononuclear Phagocytic System; (IAA), Infrarenal Abdominal Aorta; (SHAM), Sham Laparotomy; (KCB), Kupffer Cell Blockage; (AIR), Aortic Ischemia Reperfusion; (MDA), Malondialdehyde; (MPO), Myeloperoxidase; (PMNL), Polimorphism leukocytes; (TNF), Tumour necrosis factor; (IL-1), Interleukin-1; (IL-6), Interleukin-6.

**References**


Gadolinium Chloride and Aortic Occlusion-Reperfusion


