Gut Decontamination Reduces Bowel Ischemia-Induced Lung Injury in Rats*

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**Objective:** To evaluate the effects of gut decontamination on endotoxin, tumor necrosis factor (TNF) levels, and the associated lung injury in a rat model of bowel ischemia.

**Summary background data:** Gut ischemia induces disruption of the intestinal mucosal barrier, allowing translocation of bacteria and endotoxin into the blood, which may trigger a systemic inflammatory response and lung injury.

**Methods:** Thirty anesthetized rats were randomized into three groups: (1) ischemia-reperfusion (I/R) alone (a 60-min superior mesenteric artery occlusion and 4 h of reperfusion, n=10); (2) rats that underwent gut decontamination prior to ischemia (I/R+GD, n=10); and (3) control rats (sham operated, n=10). Serum levels of lipopolysaccharide (LPS) and TNF were measured at the end of the experiment. Lung permeability was measured using bovine serum albumin labeled with $^{125}$I, and organ injury was assessed histologically.

**Results:** One hour of bowel ischemia and 4 h of reperfusion (I/R) led to elevations of blood LPS and TNF levels of 0.33±0.005 EU/mL and 173±56 pg/mL, which were higher than the sham group (p<0.01). Gut decontamination (I/R+GD) significantly attenuated the LPS and TNF generation, 0.09±0.005 and 56.2±6 pg/mL (p<0.01). Lung injury as assessed by pulmonary permeability index was also reduced by gut decontamination, 2.1±0.42 vs 5.3±0.82 in the control group (p<0.03). Surprisingly no difference was detected in the number of pulmonary neutrophils in sequestration between the groups.

**Conclusions:** Our data suggest that gut decontamination can reduce the generation of LPS, TNF, and the severity of lung damage that often follows ischemia and reperfusion of the intestine in rats.

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**Key words:** endotoxemia; gut decontamination; gut ischemia; lipopolysaccharide; lung injury; tumor necrosis factor

**Abbreviations:** BALF=BAL fluid; CPM=counts per minute; I/R=ischemia-reperfusion; I/R+GD=ischemia-reperfusion with gut decontamination; LPS=lipopolysaccharide; PI=permeability index; PMN=polymorphonuclear leukocytes; TNF=tumor necrosis factor

Acute lung injury is frequently seen following intestinal ischemia-reperfusion (I/R). It has been suggested that gut ischemia induces disruption of the intestinal mucosal barrier, allowing translocation of bacteria and endotoxin from within the gut into the blood, an event that may initiate a systemic inflammatory response and the secretion and activation of inflammatory mediators, including cytokines, platelet-activating factor, and arachidonic acid metabolites. This response induces neutrophil activation and sequestration in the pulmonary microcirculation, increases endothelial and epithelial permeability, and causes acute lung injury. This proposition was reinforced by the results of previous studies that showed that intestinal ischemia-
caused release of lipopolysaccharides (LPS) into the portal vein provoked the synthesis of tumor necrosis factor (TNF). In a previous study that used a similar intestinal I/R model, we found TNF in the systemic circulation only 2 h after the beginning of the ischemic phase that peaked at 4 h, thus supporting the hypothesis that TNF is not stored in large amounts in the cytoplasmic granules, but, rather, that its secretion is dependent on its de novo transcription and release by Kupffer cells in the liver, after having been stimulated by LPS. We also showed in the same study that polyclonal antibodies and soluble TNF receptors modified the proliferation of neutrophils in the lungs, suggesting that the activation of neutrophils is partly dependent on TNF.

The purpose of the present study was to examine the effects of bowel decontamination prior to I/R on the release of endotoxin into the blood, with the subsequent secretion of TNF, and on the development of the resultant acute lung injury.

**Materials and Methods**

Adult male Sprague-Dawley rats (n=30) weighing approximately 300 g were used in the study. The animals were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals at the Tel Aviv University, and the study was approved by the Medical Center Committee on Animal Research. The rats were randomized into three equal groups: (1) I/R only; (2) I/R with gut decontamination; and (3) control. The animals were anesthetized with intraperitoneal ketamine (50 mg/kg). After a midline laparotomy was performed, collateral vessels from the inferior mesenteric artery and the celiac axis were ligated, and the superior mesenteric artery was occluded with a microvascular noncrush clip. This resulted in complete ischemia of the small bowel and the disappearance of the distal pulse. The laparotomy incision was then closed, to be opened 1 h later for removal of the clip. Reperfusion was confirmed by the return of pulsation to the mesenteric arcade. The incision was again closed and the animals were killed 4 h later. Sham-operated-on animals underwent an identical procedure, except for the occlusion of the superior mesenteric artery and its collaterals.

**Gut Decontamination**

Two days prior to the beginning of the study, the rats received clear water only and those that were selected to undergo gut decontamination also received 40 mg erythromycin as well as neomycin dissolved in 40 mL of water.

**LPS Determination**

All the experiments were done using disposable “pyrogen free” glassware, and sterile nonpyrogenic water was used for reconstitution and dilutions. Blood was sampled from the inferior vena cava and immediately placed on ice. It was then centrifuged at 3,000 g for 10 min at 4°C. The plasma was diluted 1:10 and endotoxin LPS concentration was measured using a chromogenic limulus amebocyte lysate assay (E-TOXATE; Sigma Chemical; St. Louis). The test sensitivity was 0.06 endotoxin units (EU) per milliliter.

**TNF Assay**

TNF activity was measured with a bioassay using A9 cells. The cells were seeded in 96-well microplates, 20,000 cells per well. The plates were incubated for 24 h and the supernatants were decanted. The cells were then placed on ice and human recombinant TNF (5 U/mL, 6×10^7 U/mg of protein; Genentech; San Francisco) was applied. After additional incubation on ice for 90 min, the samples were decanted and the walls were rinsed twice with cold medium. Dulbecco’s modified Eagle’s minimal essential medium containing 10% fetal calf serum and 25 mg/mL of cycloheximide was then added. Cells viability was determined at 12 h by a neutral red uptake assay.

**Histologic Assessment of Organ Injury**

The lungs of the animals that had been killed were inflated with 10% formaldehyde to a pressure of 25 cm H_2O. Following standard fixation, microthin sections were taken from the inferior aspect of each lung and were stained with hematoxylin-eosin for light microscopic analysis. Neutrophil sequestration was quantified by alveolar septal wall polymorphonuclear leukocytes (PMN). Leukocyte entrapment was expressed as the mean number of PMN per 10 high-power fields in an equivalent number of alveoli. All microscopic sections were analyzed in a blinded fashion by a pulmonary pathologist.

**Lung Permeability**

Lung permeability was assessed by using bovine serum albumin labeled with ^125^I. ^125^I albumin (0.2 mg) with an activity of approximately 1,000,000 counts per minute (CPM) in 1 mL of saline solution was injected into the inferior vena cava just prior to removing the clamp of the superior mesenteric artery. After 4 h, a midline sternotomy was carried out and 1 mL of blood was withdrawn from the right ventricle for measurement of ^125^I counts. The right lung was lavaged three times with 3.3 mL of saline solution with a total BAL fluid (BALF) return of 5 mL. The BALF ^125^I concentration was recorded. Permeability was expressed as the ratio of radioactivity in the BALF in comparison to the serum (permeability index [PI] = CPMgBALF/CPMg/serum).

Results were mean±SEM in the text and figures. Statistical significance was tested by one-way analysis of variance with Tukey and Scheffe procedures. Differences were considered significant at p<0.05.

**Results**

In the variables analysis, there was a significant difference between the groups. Tukey and Scheffe tests found differences (p<0.05) between every two groups. Reperpertination of the ischemic gut in the untreated group led to translocation of LPS and generation of TNF, as was evident by their increased serum levels 4 h after reperfusion to 0.34±0.005 EU/mL and 173±56 pg/mL vs LPS level of zero and TNF level of 17.1±2 pg/mL, respectively, in the sham-operated-on rats (p<0.05). The LPS and the TNF levels of rats that were pretreated with gut...
decontamination prior to bowel ischemia (I/R+GD) increased to only 0.09±0.005 EU/mL and 56.2±6 pg/mL, respectively, which was significantly lower than the level of the untreated group (I/R) (p<0.05) (Fig 1).

The acute lung injury, as assessed by microvasculature permeability, was also modified by the gut decontamination. The PI of the I/R group was 5.3±0.82 in comparison to 1.15±0.28 for the sham-operated-on group (p<0.05), and 2.1±0.42 for the I/R+GD group (p<0.05) (Fig 2). I/R led to lung neutrophil sequestration relative to sham-operated-on control animals (p<0.05), an event that was not significantly affected by gut decontamination (Fig 3).

**DISCUSSION**

In this study, we examined whether gut decontamination, which reduces the amount of bacteria in the bowel lumen, can modulate or prevent acute lung

**Figure 1.** LPS and TNF serum concentration (mean±SEM).

**Figure 2.** Lung permeability 4 h postreperfusion (mean±SEM).

**Figure 3.** Morphologic studies of lungs (hematoxylin-eosin, original magnification ×400), 4 h postreperfusion. Top: sham control operated demonstrating no lung injury. In both sections (center), I/R. Bottom: in I/R+GD, there is disruption of normal alveoli architecture and heavy neutrophil infiltration.
injury which often follows intestinal ischemia. While vascular occlusion results in progressive and eventually irreversible damage to the bowel itself, it is the reperfusion that provokes a severe systemic inflammatory response syndrome, acute lung injury, ARDS, and multiorgan dysfunction syndrome. Similar consequences may also follow conditions of a nonocclusive hypoperfused bowel, often seen in patients with hemorrhagic and septic shock. The gut hypoperfusion caused by intense splanchnic vasconstriction is an early response to hypotension. Wilmore and his colleagues identified the gut as a central element in the development of multiple organ failure in some postsurgical patients, due to the disruption of the gut mucosal barrier and bacterial translocation. Several studies demonstrated the conditions that accelerate this process, e.g., starvation, parenteral nutrition, alteration in the gut normal flora, and endotoxemia. As others have already demonstrated, leakage of LPS from the ischemic bowel into the systemic circulation stimulates macrophages and Kupffer cells to secrete TNF leading to lung injury. Our results clearly demonstrate that LPS serum levels were significantly lower in the gut decontamination group, probably due to the reduction of the bacterial load within the bowel.

TNF is believed to be a prime mediator in multiple organ injury via the activation of neutrophils that upregulate adhesion intergrins, the CD-11b/CD18 complex, which then interacts with endothelial cell-derived intercellular adhesion molecule-1 and then promotes adhesion of neutrophils to endothelial cells. Using a similar model, we had earlier demonstrated that TNF serum concentration peaked after 30 min of reperfusion. By blocking the biochemical activity of TNF using anti-TNF antibodies or soluble TNF receptors, we were able to modulate the lung injury after intestinal ischemia. In the present study, we were able to reduce the serum concentration of TNF by prevention of LPS translocation into the blood. This reduction of TNF is likely to be responsible for the attenuation of the lung injury. Tracey et al. had already demonstrated the relationship between the concentration of TNF and the severity of the physiologic response. When human recombinant cachectin (rHuTNF) was administered to rats in quantities similar to those produced endogenously in response to endotoxin, cachectin caused hypotension, metabolic acidosis, hemococoncentration, and death as a result of respiratory arrest. These responses were dose dependent. In our experiment, the reduction of TNF synthesis was associated with significant attenuation in lung injury as assessed by the PI. Histopathologic examination of the lungs in the high-dose group revealed occlusions of large arteries, neutrophil thrombi, migration of PMN cells through the blood vessels walls, and severe interstitial and peribronchiolar pneumonitis. These data suggest that both the neutrophils and the endothelial cells are functionally altered by TNF. This results in pulmonary microvascular inflammation with increased vasopermeability and edema formation, and enhanced neutrophil adhesion and activation, all of which release their proteolytic enzymes and reactive oxygen metabolites, leading to the development of the acute lung damage.

In our present study, we did not observe any changes in the degree of PMN sequestration within the lungs. Our results are in agreement with those of Kenton et al and Koike et al who found no relationship between the levels of either LPS or TNF and the pulmonary sequestration of PMN.

We tried to ascertain whether the reduction of endotoxemia by gut decontamination would reduce the severity of organ damage, i.e., would it reduce the accumulation of neutrophils and the microvascular leak in the lungs in this model. We have demonstrated reduction of the acute lung injury by gut decontamination that was independent of the neutrophils sequestration, as evidenced by the lower pulmonary PI in the treated rats. Our results are supported by the observation of Kenton and his colleagues who administrated both high (1.4×10^6 U/kg), and low (1.0×10^6 U/kg) doses of human recombinant cachectin to guinea pigs. They found that while the low-dose TNF did increase the pulmonary PMN sequestration, it did not change the pulmonary PI or the lung wet/dry ratio. They also observed an increased PI, without an increased left atrial or pulmonary artery pressure, indicating that the increased PI resulted from increased pulmonary microvascular permeability. Although we cannot exclude the possibility that increased pulmonary hydrostatic pressure may have affected the measurements of pulmonary permeability, it is an unlikely speculation since no fluids were administered to the rats during the experiment, and reduced cardiac function does not occur after such a short experiment.

In summary, we demonstrated that a simple procedure, such as gut decontamination, can effectively reduce the amount of endotoxin released into the systemic circulation from an ischemic gut. Gut decontamination also minimized TNF synthesis and attenuated lung injury.

As previously reported, LPS and TNF levels rise following bowel manipulation and aortic clamping in patients undergoing abdominal aortic surgery. It is possible that by performing gut decontamination in
patients with low-flow states, shock, and gut ischemia, some of the pulmonary complications that may follow can be prevented.

REFERENCES