N-Acetylcysteine Attenuates Bacterial Translocation after Partial Hepatectomy in Rats

Erdem Okay, M.D.,*,1 Aynur Karadenizli, M.D.,† Bahar Müezzinoglu, M.D.,‡ Umit Zeybek, Ph.D.,§ H. Arzu Ergen, M.Sc.,§ and Turgay Isbir, Ph.D.§

*Department of General Surgery, †Department of Microbiology and Clinical Microbiology, and ‡Department of Pathology, Kocaeli University School of Medicine, Derince/Kocaeli, Turkey and §Department of Molecular Medicine, Istanbul University Institute of Experimental Medical Research, Istanbul, Turkey

Submitted for publication October 18, 2004

Background. Translocating enteric bacteria have been suggested as playing a major role in the development of infections after partial hepatectomy. We investigated the effect of N-acetylcysteine (NAC) on bacterial translocation (BT) and intestinal mucosa as the first line of defense against BT.

Materials and methods. We compared four groups of eight Sprague–Dawley male rats each: sham, control (partially hepatectomized), partial hepatectomy plus preoperative single-dose NAC, and a fourth that received partial hepatectomy with a preoperative single-dose NAC plus treatment with NAC for 2 days. Microorganism counts of tissues, lung injury score, lung tissue glutathione, and malondialdehyde levels and microscopy of intestinal mucosa were studied at the end of 48 h.

Results. Microorganism count in the lung and mesenteric lymph node cultures and lung injury score were significantly higher in the control group when compared with the sham, third, and fourth groups (lung: 9919.6 versus 0.0, 2912.9, 1550.0 cfu/g tissue; mesenteric lymph nodes: 8458.3 versus 0.0, 89.0, 88.9 cfu/g tissue; lung injury score: 3.25 versus 0.5, 1.13, 1.75). In the control group, the villous height of the distal ileal mucosa was significantly shorter than the sham group (65.25 versus 75.25 μm) and the difference from groups 3 and 4 was not statistically significant. Neutrophil infiltration in the distal ileal mucosa of the control group was significantly higher than the sham, third and fourth groups (3.13 versus 0.25, 0.38 and 1.0).

Conclusions. The parenteral use of NAC attenuates bacterial translocation after partial hepatectomy in rats. Attenuation of the lung injury after partial hepatectomy in NAC-treated groups might be attributable to both anti-inflammatory effect and the effect on BT. © 2005 Elsevier Inc. All rights reserved.

Key Words: bacterial translocation; hepatectomy; acetylcysteine; rat; experimental.

INTRODUCTION

Partial hepatectomy is performed frequently for tumors, trauma, and other problems in surgical practice. Bacterial infections are major causes of postoperative morbidity and mortality after partial hepatectomy [1, 2]. In clinical and experimental studies, enteric bacteria have been suggested to play major role in the development of these infections [1, 3]. Bacterial overgrowth in colon and distal ileum has been blamed as the primary step of the bacterial translocation (BT) [4]. These microorganisms invade through the enteroctyes and then translocate into mesenteric lymph nodes (MLN), lung, and the other organs [1, 4–7]. The first line of defense against BT is the intestinal mucosal barrier. There are various studies about the impairment of this defense mechanism after hepatectomy and other conditions [8–13]. Disturbed microcirculation of the terminal ileum and local tissue hypoxia, ultrastructural impairment of epithelial cells, overactivated macrophages and neutrophils, and radical oxygen species (ROS) are some of the investigated factors affecting BT.

N-acetylcysteine (NAC) is a thiol-containing compound that protects cells against oxidative damage by reacting directly with ROS as a direct antioxidant and by increasing the cytoplasmic reserve of glutathione (GSH) [14–16]. In addition, NAC has an anti-inflammatory action and reduces the ability of neutro-
phils and monocytes to generate and release ROS [17–19]. Improvement of mesenteric and portal circulation with the application of NAC in various conditions has been investigated by several authors [20–23]. The parallelism of the influences of NAC and the investigated mechanisms of mucosal barrier impairment compelled us to study the effects of NAC on BT after partial heptectomy.

MATERIALS AND METHODS

Animals

The protocol of the study was approved by the Research Ethics Committee of Istanbul University Institute of Experimental Medical Research. Sprague–Dawley male rats weighing between 240 and 290 g were used for the study. Rats were housed in stainless-steel cages (three rats in each case) under 12-h day–night cycle at constant temperature of 21°C during the study and were fasted overnight before surgery without water restriction. Anesthesia was performed with the intraperitoneal administration of ketamine hydrochloride (50 mg/kg), and rats inhaled the room air during anesthesia.

Experimental Design

Four groups were designed, each having eight rats: the first (sham) group had laparotomy and dissection of hepatic ligaments only; the second (control) group had partial heptectomy; the third group had partial heptectomy and preoperative single dose injection of 400 mg/kg NAC (Asist ampule 100 mg/mL, Husnu Arsan İlaçları A.S, Turkey); and the fourth group was the same as the third group except that treatment with NAC 150 mg/kg-day (qid) for 2 days was added.

Surgical Procedure

In groups 3 and 4, 400 mg/kg NAC was injected over the course of 5 minutes (approximately 0.2 mL/min) via the penile vein after anesthesia. The abdominal surface was prepared for operation, and the abdomen was explored with a midline incision below xyphoid process (20 min after the NAC injection in groups 3 and 4). Left anterior and median lobes of the liver were removed (approximately two-thirds heptectomy) after the dissection of the ligaments and ligation of the proximal vessels of these lobes, according to the technique described by Higgins and Anderson [24]. Four to five milliliters of 0.9% NaCl was injected into the abdominal cavity before the closure. Fascia and skin were closed separately with 3-O polypropylene. Rats were allowed to drink water and eat rat pellets postoperatively. Only ligaments of liver were dissected in the sham group. In group 4, 150 mg/kg-day (qid) NAC was injected intramuscularly to each rat during postoperative 48 h.

Rats were sacrificed with cervical dislocation at the end of the 48 h. The abdomen and thorax were opened, and approximately 0.15–0.20 g of lung tissue was taken from the right lower lobe of the lung under sterile conditions and put into a 10% neutral-buffered formaldehyde solution for pathological examination. The average number of microorganisms in cultures of tissues is shown in Table 1. There was no bacterial growth in cultures of lung and MLN in the sham group. The logarithmic transformed value of bacterial count in the lung and MLN cultures were significantly higher in the control group when compared
with the sham group and groups 3 and 4. *Escherichia coli* and *Enterococcus faecalis* were the isolated microorganisms.

Histologically, there was no evidence of lung injury in the sham group. However, lung injury score was significantly higher in the control group when compared with the sham group and groups 3 and 4. The detailed analysis of histological lung injury score is shown in Table 2.

GSH and MDA values of the lung tissue were significantly higher than the sham group compared with the control group. GSH value was slightly higher, and MDA values were slightly lower in groups 3 and 4 when compared with the control group (Table 3).

In the control group, the villous height of the distal ileal mucosa was significantly shorter than the sham group. There was a slight increase of villous height in groups 3 and 4, but the difference was not statistically significant. Neutrophil infiltration in the distal ileal mucosa of the control group was significantly higher than the sham group and groups 3 and 4 (Table 4). We did not observe necrotic foci in the mucosa, subepithelial space, denuded villi or epithelial desquamation, submucosal edema, or disintegration of lamina propria, which have been mentioned in the literature as the other indicators of mucosal barrier impairment [28, 29].

**DISCUSSION**

Intraluminal bacterial overgrowth, adherence to the epithelial surface, and translocation through the enterocytes as the first steps of BT have been investigated by several authors [4, 6, 30]. It has been suggested that the major route of BT is lymphogenous and that the lymphatic–thoracic duct systemic circulation might play a motor role in BT [31, 32]. We also detected microorganisms in MLN and lung tissue attributable to BT after partial hepatectomy. However, Wells *et al.* have claimed that macrophages were responsible for the passage of bacteria to extraintestinal sites by the way of phagocytosis [33]. According to this theory, bacteria are transported to an extraintestinal site by the macrophages after phagocytosis in the bowel wall. Having failed to degrade, the bacteria are released free in the extraintestinal site after death of the phagocytic cell, thus resulting in BT.

Some studies have shown that major liver resection results in a reduction in intestinal microcirculation and alterations in intramucosal pH and oxygen extraction of the gastrointestinal tract [12, 13]. These authors have concluded that the opening of arteriovenous shunts and the diversion of blood away from the gastrointestinal tract might cause hypoxia. Thies *et al.* [21] observed significantly better portal vein flow in transplanted patients treated with NAC. In the study of Schmidt *et al.* [22], treatment with NAC increased the mesenteric postcapillary flow of the rabbits that received *E. coli* lipopolysaccharides. Zhang *et al.* [22] also showed that NAC increased mesenteric arterial flow. Kigawa *et al.* [23] showed that NAC improves portal flow when applied to rats with raised portal resistance attributable to bile duct ligation. All these studies imply that improvement on mesenteric circulation with NAC treatment might have a role on intestinal microcirculation and mucosal barrier.

### Table 1

**Count of Microorganisms (Colony-Forming Units per Gram) in MLN and Lung Tissue**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Control</th>
<th>Single dose of NAC</th>
<th>NAC treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microorganisms in MLN</td>
<td>0.0 ± 0.0</td>
<td>9919.6 ± 3049.1 (** s)</td>
<td>2912.9 ± 2471.3 (** c)</td>
<td>1550.0 ± 1909.6 (** c)</td>
</tr>
<tr>
<td>Microorganisms in lung</td>
<td>0.0 ± 0.0</td>
<td>8458.3 ± 4679.9 (** s)</td>
<td>89.0 ± 83.4 (c)</td>
<td>88.9 ± 156.6 (c)</td>
</tr>
</tbody>
</table>

*Note. Values in the cells are presented as mean value ± standard deviation. The value is significantly (* P ≤ 0.05; ** P ≤ 0.001) lower (\(\sim\)) or higher (\(\sim\)) from sham (s) or control (c) group.*
TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Control</th>
<th>Single dose of NAC</th>
<th>NAC treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Tissue GSH (nmol/g wet weight)</td>
<td>18.45 ± 2.2</td>
<td>25.99 ± 4.95 (⁎⁄⁄ s)</td>
<td>27.16 ± 2.03 (⁎⁎⁄⁄ s)</td>
<td>27.86 ± 2.59 (⁎⁎⁎⁄⁄ s)</td>
</tr>
<tr>
<td>Lung Tissue MDA (μg/g protein)</td>
<td>0.39 ± 0.07</td>
<td>0.57 ± 0.07 (⁎⁄⁄ s)</td>
<td>0.52 ± 0.06 (⁎⁎⁄⁄ s)</td>
<td>0.48 ± 0.12</td>
</tr>
</tbody>
</table>

*Note. Values in the cells are presented as mean value ± standard deviation. The value is significantly (⁎ P ≤ 0.05; ⁊⁎ P ≤ 0.001) lower (⁄⁄) or higher (⁎/) from sham (s) or control (c) group.*

Wang et al. [34] observed an increase in permeability of the capillary endothelial membrane and blood–tissue barrier of the intestine after major liver resection. They noticed that compromised junction of intestinal epithelial cells allowed the passage of tracers and their appearance in-between the cells. Failure of the intestinal blood–tissue barrier has been implied by an increase in permeability of endothelial cell membranes and capillaries, resulting in leakage of blood components in a local inflammatory response, associated with gut barrier failure and enteric bacterial invasion after major liver resection. The study of Alexandris et al. [35] might explain the problem in a molecular manner. They have shown that protein oxidation has increased after partial hepatectomy and they suggest that this event could change the structure and function of the tight junction-associated proteins. NAC might be blocking the disruption of the paracellular barrier possibly by the mechanism explained above and therefore attenuating intestinal mucosal barrier dysfunction.

Spapen discussed in detail, the value of NAC in a recent commentary [36]. Although clinical [37] and experimental [38] studies could not show any benefit, there are many studies stating that NAC improves the problems associated with sepsis. In experimental studies, NAC attenuates endotoxin-induced alterations in leukocyte–endothelial cell adhesion and macromolecular leakage [39] and has been shown to have an anti-inflammatory and antiapoptotic effect in lungs in a sepsis model of cecal ligation and puncture [40]. In clinical studies, NAC has been shown to attenuate the peroxidative stress when applied in severe sepsis and early stage of septic shock [41], to block the release of tumor necrosis factor alpha and interleukin-8 [42], and to enhance cardiac output and tissue oxygenation [43]. Spapen also concluded that timing and dosing might be important for the benefit of NAC and that further studies are needed to define the real value of NAC as an adjuvant therapeutic in sepsis.

Hoffer et al. [18] demonstrated that the administration of NAC inhibits the release of chemotactic factors for neutrophils and reduces neutrophil infiltration in the lungs of intoxicated animals when measured 24 h after the administration of paraquat. Anti-inflammatory action of NAC-suppressing cytokine expression-release was also reported by Tsuji et al. [19].

Histopathological changes of intestinal mucosa after partial hepatectomy include villous edema, a decrease in villous height, and infiltration of inflammatory cells [29, 32, 34]. Neutrophils are the first migrating cells to the inflammatory region and responsible from the early stages of phagocytic action. Subsequently, monocytes differentiate to tissue macrophages and become the predominant cell type. Although the relationship between BT and macrophage has been investigated by the aforementioned authors, there are studies about neutrophils and BT. Kubes et al. discussed the changes in gut permeability with the release of proteolytic enzymes and ROS from neutrophils leaking out of the microvasculature [44]. Fazal et al. have emphasized the increased infiltration of activated neutrophils into the intestinal tissue after burn injury causing enhanced intestinal BT [45]. Depending on our findings and previous reports, it might be speculated that the direct and indirect effects of NAC on ROS derived from inflammatory cells might have attenuated the impairment of mucosal barrier. Furthermore, NAC also might be effective against BT by influencing the role of inflammatory cells in translocation action.

Koch et al. [46] reported that the administration of 250 mg/kg NAC to rabbits was associated with a suppressed polymorphonuclear leukocyte oxidative burst.

TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Control</th>
<th>Single dose of NAC</th>
<th>NAC treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal mucosa villous height (μm)</td>
<td>75.25 ± 3.95</td>
<td>65.25 ± 4.17 (⁎⁄⁄ s)</td>
<td>66.38 ± 4.66</td>
<td>68.63 ± 4.66</td>
</tr>
<tr>
<td>Count of mucosal neutrophil infiltration</td>
<td>0.25 ± 0.46</td>
<td>3.13 ± 2.03 (⁎⁎⁄⁄ s)</td>
<td>0.38 ± 0.52 (⁎⁎⁄⁄ c)</td>
<td>1.00 ± 1.20 (⁎⁄⁄ c)</td>
</tr>
</tbody>
</table>

*Note. Values in the cells are presented as mean value ± standard deviation. The value is significantly (⁎ P ≤ 0.05; ⁊⁎ P ≤ 0.001) lower (⁄⁄) or higher (⁎/) from sham (s) or control (c) group.*
activity, resulting in a delayed bacterial clearance and enhancement of organ colonisation in liver, lung, and kidney after the intravenous injection of E. coli. They suggested that high doses of NAC impaired the potency of the granulocyte-dependent killing of the bacteria, which seems contradictory to our findings. However, we did not perform this study with injected microorganisms, and the decrease of the bacterial count found in MLN and lungs were attributed to attenuated BT.

In this study, we detected that lung injury may occur in a model of partial hepatectomy without ischemia/reperfusion and that NAC has a remedial effect on this injury. However, we do not have enough data to state whether the observed benefit is attributable to a decrease of BT or direct protective effects of NAC on lung tissue or both.

In their study using an ex vivo blood-free model, Weinbroum et al. [47] concluded that NAC has dual effect on lung tissue after liver ischemia/reperfusion: NAC replenishes the lung's GSH content, thus augmenting lung antioxidant potency, and has a direct role in limiting the build-up of hepatic xanthine oxidase, which is cytotoxic for endothelial cell and induces increased microvascular permeability and, subsequently, ROS, within the lung. It has been observed that higher doses of NAC compared with lower doses have lesser but almost equally protected lung tissue despite the lower GSH content. There are other studies proving the effect of NAC as a direct scavenger of ROS in the lung [48, 49].

Despite the definite histological changes, statistically insignificant difference of lung tissue GSH values might be a clue for a mechanism other than the replenishment of GSH. The dosage of NAC preferred in this study might be the reason of this unexpected slight increase. Weinbroum et al. [47] have determined that vasodilatation and oxygen availability related to the higher dose of NAC could augment the influx of molecular oxygen into the lung, thereby providing more abundant formation of ROS. They concluded that this may therefore upset the NAC scavenging activity of ROS and the replenishing of the GSH pool.

As an indicator of lipid peroxidation, MDA values of lung tissue increased in our study as a result of lung injury after partial hepatectomy. Insignificant decrease of MDA levels in both NAC groups might be valuable when considered together with the lung injury score. We assume that application of NAC (especially single dose) is much more effective on septal edema and infiltration of inflammatory cells than the injury of the lung parenchymal cells where lipid peroxidation occurs. Besides, inflammatory cells are the source of ROS that cause injury on pulmonary tissue.

In conclusion, parenteral use of NAC attenuates bacterial translocation after partial hepatectomy in rats. Although more experimental and clinical studies are needed, this multipurpose drug should be considered for this postoperative problem. Our finding of attenuation of the lung injury after partial hepatectomy in NAC-treated groups, which is consistent with previous studies in the literature, might be attributable to both anti-inflammatory effect and the effect on BT.

It is likely that the attenuating effect of NAC on BT will be reflected in the medical literature as a part of the discussion about the role of BT on multiple organ dysfunction syndrome and sepsis. Earlier and later phases of the translocation process after partial hepatectomy in the event of NAC application might be the aim of further studies. Ultrastructural and molecular investigations would reveal the exact mechanism of action of NAC on intestinal mucosal barrier and translocation process.

ACKNOWLEDGMENT

We thank the Istanbul University Institute of Experimental Medical Research for allowing us to do this experiment in their laboratory.

REFERENCES


