Hemorrhagic Shock and Bacterial Translocation

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ABSTRACT

Background: Bacterial translocation (BT) is increased under some circumstances. It is believed that xanthine oxidase generated oxidants may play a major role in promoting bacterial translocation by disrupting mucosal barrier function in hemorrhagic shock. The aim of this study is to determine the effects of free oxygen radicals on hemorrhagic shock induced bacterial translocation, and phagocytic burst activity status after shed blood resuscitation.

Material and Methods: We studied the effects of free oxygen radicals in hemorrhagic shock induced intestinal mucosal injury and its role on bacterial translocation, and nonspecific host immune defense status in hemorrhagic shock in 36 male albino rats. In the control group (group I, n = 12 rats), rats were not exposed to any manipulation. In the sham group (group II, n = 12 rats), rats were catheterized, but blood was not withdrawn. In the shock group (group III, n = 12 rats), rats were subjected to 30 minutes of hemorrhagic shock (mean arterial pressure, 30-35 mmHg) followed by reinfusion of shed blood. Twenty-four hours after hemorrhage and reinfusion, the mesenteric lymph node, liver, spleen, peritoneum, cecum, and blood samples were harvested from each animal for bacterial culture. Ileum were examined histopathologically, ileal mucosas were examined biochemically, and blood samples were examined immunologically.

Results: The weights were similar in all groups, and no difference in both gram-negative and total number of bacteria found in cecal stool cultures between all groups was found. Bacterial translocation was observed in group III (25%). Tissue glutathione reductase (GR), Glutathione-S transferase (GST) levels, as a free oxygen radical indicator, were higher in group III than in groups I and II (P < 0.05). Epithelial pathological changes observed in group III were significantly greater than the other groups (P < 0.05), and phagocytic burst activity was significantly increased in group III (P < 0.05).

Conclusion: Hemorrhagic shock promotes bacterial translocation by means
of free oxygen radicals that produce epithelial changes, and shed blood resuscitation increases phagocytic burst activity.

**INTRODUCTION**

Despite technological development, posttraumatic and postoperative sepsis still remain major causes of morbidity and mortality.\(^1\) Clinical and experimental studies have shown that indigenous bacteria can translocate to systemic organs and cause systemic infections, a process termed bacterial translocation (BT).\(^4\)\(^5\) BT is increased under certain circumstances such as hemorrhagic shock,\(^6\)\(^7\) antibiotic therapy,\(^8\)\(^9\) total parenteral nutrition,\(^10\)\(^11\) intestinal obstruction,\(^12\) thermal injury,\(^13\) malnutrition,\(^14\) elemental diets,\(^15\) cytotoxic drugs,\(^16\) etc. It is believed that xanthine oxidase generated oxidants may play a major role in promoting bacterial translocation by disrupting mucosal barrier function in hemorrhagic shock.\(^9\)\(^10\)\(^14\)\(^24\) The aim of this study is to determine the effects of free oxygen radicals on hemorrhagic shock induced bacterial translocation, and phagocytic burst activity status after shed blood resuscitation.

**MATERIAL AND METHODS**

In this study, male albino rats weighing 240 to 350 g were maintained following the guidelines of the Surgical Research Committee of Ege University. Rats were brought to our Surgical Research Laboratory one week prior to experiment in order to adapt to the environment and were fed with rat chow regularly. Animals were randomly assigned to three groups, a control group (group I, \(n = 12\)), sham group (group II, \(n = 12\)), and shock group (group III, \(n = 12\)).

**Shock Model**

The rats were anesthetized with intramuscular injection of 75 mg/kg ketamine sulfate. The right carotid artery of each animal was isolated by sterile techniques through a longitudinal incision and cannulated with polyethylene tubing that contained heparinized saline solution (10 units per milliliter). A three-way stopcock was attached in-line for withdrawing blood and for monitoring blood pressure (MDE ESCORT, Model E100, Serial 5749, Medical Data Electronics, Arleta, Calif). Blood was withdrawn into a syringe that contained heparinized saline solution until the mean arterial pressure (MAP) was reduced to 30 to 35 mm Hg. MAP was maintained at 30 to 35 mm Hg for 30 minutes by withdrawal as needed. At the end of the shock period, the withdrawn blood was rein infused. Once the rats recovered from the anesthesia, they were allowed immediate access to food and water ad libitum. The sham group rats were anesthetized and their right carotid arteries were cannulated, but no blood was withdrawn, and basal MAP were obtained and cannula was removed after 30 minutes.

**Microbiological Assessment**

The rats in groups II and III were sacrificed 24 hours after shock or sham shock, the rats in group I, were also sacrificed concomitantly, and their organs were tested for translocating bacteria. Under sterile conditions, a midline abdominal incision was made and, mesenteric lymph nodes (MLNs), spleen, liver, and cecum were removed; a 0.5 mL blood sample was drawn from the inferior vena cava, and the peritoneal cavity was cultured for quantitative cultures of translocating bacteria. The organs were weighed and homogenized and blood and aliquots were prepared for aerobic and anaerobic cultures. The types and quantitative amounts of bacteria per gram tissue were estimated 24 to 48 hours later. Gram-negative enteric bacteria were identified by API system (ID 32E, BioMerieux, Marcy l’Etoile, France) and
Table 1. Bacterial Species Cultured in Shock Group

<table>
<thead>
<tr>
<th></th>
<th>E coli</th>
<th>P vulgaris</th>
<th>P mirabilis</th>
<th>K oxytaca</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLN</td>
<td>3/3</td>
<td>1/3</td>
<td>1/3</td>
<td>1/3</td>
</tr>
</tbody>
</table>

'MNL indicates mesenteric lymph node.

the other standard methods were also used for identification. Presence of 100 or more bacteria per gram tissue was accepted as the criteria for translocation.

Pathological Assessment
A 5 cm distal ileal segment from the ileocecal junction was resected for each animal, the lumen was irrigated with sterile saline and the wall of the segment was transsected from the antimesenteric border for pathological analysis. The ileal segments were analyzed pathologically by light microscopy and 5 different villus lengths were measured for each specimen after the average villus length was calculated.

Biochemical Assessment
One more 5 cm ileal segment was also resected, irrigated, and opened from antimesenteric border, as mentioned above, from each animal. From each segment, 200 mg mucosa was obtained and preserved at -80°C prior to analysis. The tissues were homogenized on ice at 1,500 rpm for 2 minutes in a buffer containing 50 mM TRIS, 0.1 mM EDTA, pH 7.60. To yield the cytosolic fractions, the homogenates were centrifuged at 30,000 g for 60 minutes at 4°C. Tissue enzymes and protein analyses were performed within 8 hours in the supernatants preserved at 4°C. Protein determinations were performed according to Lowry et al. Superoxide dismutase (SOD) and glutathione peroxidase (GSH Px) activities were determined using Randox kits (Randox Laboratories Ltd, Crumlin, UK). Glutathione reductase (GR) and glutathione-S transferase (GST) activities were measured by modified methods. All determinations were carried out on the Hitachi 704 automatic analyzer (Hitachi Ltd., Tokyo, Japan). Tissue enzyme results were expressed as mU/mg protein.

Immunological Assessment
One milliliter of blood obtained by cardiac puncture was withdrawn into a syringe that contained heparinized saline solution. This blood sample was subjected to flow cytometric (Cell-Quest software, BD Biosciences, Calif) evaluation of macrophage burst activity by using Bursttest (Phagoburst, Orpegen Pharma, Heidelberg, Germany) kits for each animal.

Statistical Analysis
Statistical analysis was performed using SPSS for windows program and translocation incidences were evaluated by chi square analysis. Data about weights, enzyme levels, macrophage burst activity levels, and villus lengths were analyzed by using Kruskal-Wallis variance analysis (ANOVA) and Mann-Whitney U tests.

RESULTS
Bacterial translocation did not occur in the control and sham groups, although bacteria translocated to the MLN (25%) of rats subjected to hemorrhagic shock. The most common bacterial species cultured from shocked rats were E coli;
Table 2. Incidence of Bacterial Translocation and Sites, and Cecum Bacteria Population

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>MLN</th>
<th>Liver</th>
<th>Spleen</th>
<th>Cecum population (col U/g)</th>
<th>E coli</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0/12</td>
<td>0/12</td>
<td>0/12</td>
<td>3.31 x 10^6 ± 2.43 x 10^6</td>
<td>3.57 x 10^6 ± 2.43 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>12</td>
<td>0/12</td>
<td>0/12</td>
<td>0/12</td>
<td>3.13 x 10^6 ± 1.00 x 10^6</td>
<td>3.39 x 10^6 ± 1.06 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td>12</td>
<td>3/12</td>
<td>0/12</td>
<td>0/12</td>
<td>3.45 x 10^6 ± 1.65 x 10^6</td>
<td>3.60 x 10^6 ± 1.78 x 10^6</td>
<td></td>
</tr>
</tbody>
</table>

P > 0.05  P > 0.05  P > 0.05

Table 3. Pathologic Evaluation of Ileal Segments

<table>
<thead>
<tr>
<th>Group</th>
<th>subepithelial edema</th>
<th>subepithelial disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Medium</td>
</tr>
<tr>
<td>Control</td>
<td>5/12</td>
<td>3/12</td>
</tr>
<tr>
<td>Sham</td>
<td>4/12</td>
<td>3/12</td>
</tr>
<tr>
<td>Shock</td>
<td>4/12</td>
<td>4/12</td>
</tr>
</tbody>
</table>

P < 0.05  P < 0.05

however, other bacteria accompanied *E. coli*, including *Proteus sp* and *Klebsiella* (Table 1). The cecal population levels of bacteria were not statistically different between control, shocked, and sham shocked rats (P > 0.05) (Table 2).

The ileal mucosa of the rats subjected to hemorrhagic shock, but not sham-shocked and control animals, had significantly more severe subepithelial edema and epithelial disruption (P < 0.05) (Table 3) (Figure). There were significant changes in villus lengths of the rats subjected to hemorrhagic shock compared to control and sham-shocked rats’ villus lengths (P < 0.05) (Table 4).

SOD levels were higher in group III than in groups I and II, there was no difference statistically (P > 0.05), however, GR and GST levels were significantly higher in group III than in groups I and II, respectively (P < 0.05, P < 0.01) (Table 5).

Phagocyte burst activity levels, sampled from whole blood obtained in the post-shock period, were higher in the shock group when compared to the control and sham groups, respectively (P < 0.05) (Table 6).

**DISCUSSION**

Hemorrhagic shock is a form of hypovolemic shock, and is a major problem in intensive care units after trauma and postsurgery. It is a severe insult that directly affects the organism by means of systemic changes. These changes can severely disrupt the functions of the organism, and ultimately be lethal.

Under normal circumstances the gut functions to absorb nutrients and to exclude luminal bacteria and their products. Recent studies have documented that bacteria normally contained within the gut can cross the mucosal barrier and cause systemic infections, a process termed bacterial translocation.\(^4,10,30\)

Invasion of peritoneal cavity by bacteria from organs either covered by or adjacent to the peritoneum was reported in the late 19th and early 20th centuries.\(^5\) In 1891, it was demonstrated in vivo that viable bacteria could pass through an intact intestinal wall, in 1895, it was
reported that gram-positive cocci passed unaided through intact intestinal mucosa. Bacterial translocation is defined as the passage of viable bacteria through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes, and possibly other tissues. This concept was refined to include all microbial translocation, defined as the passage of both viable and nonviable...
microbes and microbial products such as endotoxin across an anatomically intact intestinal barrier.6

On the other hand, whether there is a relationship between shock and the development of bacteremia/endotoxemia has been discussed since the 1950’s when Fine et al proposed that the gut could be a reservoir for bacteria causing systemic infections in surgical patients.8,11,35 Furthermore, longer periods of hypotension had both a higher mortality and a higher incidence of bacterial translocation.8,10,11,13 This suggests that there may be a relationship between the duration of the shock period and the incidence and magnitude of bacterial translocation.8

Factors that promote the translocation of bacteria from the gastrointestinal tract include: a) disruption of the normal ecological balance of the indigenous microflora resulting in bacterial overgrowth,14,17,21 b) impaired host immune defenses,15,16,18,22,32 and, c) physical disruption of the intestinal mucosa barrier to bacteria.8,13,15,16,22 In our study, the incidence of bacterial translocation to the MLN of shocked rats was higher (3/12, 25%), but was not significantly different from control rats and rats subjected to sham. Translocated bacteria in the shock group were the same with cecal population showing that an ecological imbalance, which would have lead to colonization of more pathogenic microflora, did not develop. Additionally, organisms cultured from cecum, quantitatively, were not different for each group when compared. These findings suggest that no changes occur in the ecological equilibrium in the intestinal microflora during hemorrhagic shock, and changes on the other two mechanisms may lead bacterial translocation in hemorrhagic shock. Thus, the differences in translocation incidences were not related to a shock-induced disruption of the normal ecological balance of the indigenous gastrointestinal microflora.

In previously mentioned studies, it was shown that the most translocating bacteria are endogenous gram-negative microorganisms, and translocation mostly occurs in distal ileum and cecum.8,31 In the present study, similarly, gram-negative microorganisms were the main translocated bacteria.

After the microscopic examinations of the terminal ileal mucosas; the lengths of intestinal villus were significantly shorter; additionally, subepithelial edema was significant in the hemorrhagic shock group when compared to the others. While there was separation of the epithelium from basal lamina and epithelial disruption, particularly at the villus tips in the hemorrhagic shock group, these findings were not observed in other groups. These findings suggest that the intestinal defense barrier disruption plays a significant role in bacterial translocation in hemorrhagic shock.

Depending on the duration of hemorrhagic shock, the degree of intestinal mucosal disruption, which is the result of ischemia, increases and plays the main role in bacterial translocation.8,30 In consequent reports, it is postulated that epithelial disruption occurs, not only due to ischemia, but also due to the effect of protease or free oxygen radicals in the epithelium.8,9 Competitive inhibition or inactivation of xantine oxidase, which plays the main role in ischemia reperfusion injury, showed that intestinal mucosal damage decreases.9,33,34 Thus, limited periods of hemorrhagic shock

### Table 4. Average Lengths of Villus

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean lengths</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>294.1 ± 60.9 µm</td>
</tr>
<tr>
<td>Sham</td>
<td>320.9 ± 43.3 µm</td>
</tr>
<tr>
<td>Shock</td>
<td>247.5 ± 71.4 µm</td>
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<table>
<thead>
<tr>
<th>P</th>
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<td>&lt; 0.05</td>
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appear to promote bacterial translocation by injuring the gut mucosa and impairing its barrier function. Free oxygen radicals are originally in vivo side products of normal metabolism and potentially reactive molecules. The most important molecules are superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl (OH). There are more free oxygen radical origins, but there are also more defense or scavenger mechanisms in organisms, such as complex enzyme systems, namely superoxide dismutase (SOD), katalase, glutathione peroxidase (GSH Px), glutathione reductase (GR), glutathione S-transferase (GST), etc. Since recent studies have implicated xantine oxidase generated oxygen free radicals as the mediators of tissue injury in ischemia-induced intestinal injury, we investigated whether intestinal tissue damage is due to xantine oxidase generated free oxygen radicals or not, and for this aim, as an indirect indicator of presence of oxygen radicals, in the mucosa of the distal ileum tissue enzymes, namely SOD, GR, GSH Px, and GST levels were obtained. SOD levels were higher in group III than in groups I and II, but not statistically different ($P > 0.05$). On the other hand, GR and GST levels were significantly higher in group III than in groups I and II, respectively ($P < 0.05, P < 0.01$). These findings suggest oxygen-derived radicals may play a role in intestinal mucosal disruption leading to bacterial translocation in hemorrhagic shock.

In previously reported papers, it was stated that bacterial invasion leading to posttraumatic sepsis is believed to be secondary to trauma, and/or hemorrhagic shock-induced immune suppression. Trauma combined with hemorrhage produces more protracted impairment in cell-mediated immunity than trauma or hemorrhage alone. Hemorrhage without tissue trauma produces immunosuppression and enhances susceptibility to sepsis, increases susceptibility to infection after sustained hemorrhage, and results in impaired neutrophil function. It is believed that these changes cause bacterial translocation, which causes irreversible changes. Phagocytosis is an important antifungal and antibacterial function of a healthy organism that is a branch of nonspecific immune defense mechanisms. Phagocytic burst activity is also the main and final function of this process. There are few experimental studies on the status of phagocytic burst activity in hemorrhagic shock models.

<table>
<thead>
<tr>
<th>Group</th>
<th>Phagocyte burst activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.35 ± 18.35</td>
</tr>
<tr>
<td>Sham</td>
<td>55.99 ± 27.14</td>
</tr>
<tr>
<td>Shock</td>
<td>80.54 ± 14.22</td>
</tr>
</tbody>
</table>

*Table 5. Enzyme Levels of Mucosa of Distal Ileum*

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (mU/mgprot)</th>
<th>GR (mU/mgprot)</th>
<th>GSH Px (mU/mgprot)</th>
<th>GST (mU/mgprot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2186.2 ± 237.9</td>
<td>371.1 ± 44.9</td>
<td>245.1 ± 44.2</td>
<td>45.2 ± 11.7</td>
</tr>
<tr>
<td>Sham</td>
<td>2291.7 ± 357.5</td>
<td>326.9 ± 50.5</td>
<td>224.3 ± 32.2</td>
<td>49.2 ± 15.6</td>
</tr>
<tr>
<td>Shock</td>
<td>2338.0 ± 294.3</td>
<td>425.3 ± 68.7</td>
<td>231.8 ± 76.1</td>
<td>89.9 ± 54.1</td>
</tr>
</tbody>
</table>

$P > 0.05$ $P < 0.05$ $P > 0.05$ $P < 0.01$

*SOD indicates superoxide dismutase; GR, glutathione reductase; GSH Px, glutathione peroxidase; and GST, glutathione-S-transferase.*
Rhee et al\(^8\) reported that neutrophil activation increases significantly immediately after hemorrhage, and it is greatest after resuscitation with lactated Ringer’s (LR) solution, but not after resuscitation with shed blood and hypertonic saline. They summarize that these findings suggest that neutrophil activation may be caused by LR and not by reperfusion. In our study we investigated phagocytic burst activity in the 24 hours post-shock, and results suggested that burst activity was significantly higher in the hemorrhage group animals that received shed blood when compared to control and sham group animals, respectively (\(P < 0.05\)). Bacterial translocation with or without its endotoxins may be the cause of an increase of neutrophil activation; activated neutrophils can adversely affect the hemorrhaged organisms through production of superoxides. Fuller et al showed that bacterial translocation is decreased through nonspecific immune response increasing in animals that received \textit{Propionibacterium acnes}.\(^5\)

These findings support our study, in which we found that neutrophil burst activity significantly increases, but bacterial translocation is not statistically significant in hemorrhagic shocked animals. Deitch et al\(^1\) showed that in neutrophil depleted animals bacterial translocation increases as much as intestinal mucosal damage in hemorrhagic shock and speculate that neutrophils are not responsible for intestinal mucosal damage, although they play major role in killing the translocated bacteria. Our study is consistent with these results. In our study, intestinal mucosal damage and neutrophil burst activity are elevated, but bacterial translocation is not significant in hemorrhaged and reperfused animals.

As a result, bacterial translocation occurs after intestinal mucosal injury induced by ischemia reperfusion, and neutrophil burst activity, an indicator of host nonspecific immune response, is elevated during a long period on post-shock. Shed blood resuscitation increases neutrophil burst activity. Translocated bacteria may be inactivated by neutrophil burst activity.

**CONCLUSION**

The cause of bacterial translocation in hemorrhagic shock is ischemia reperfusion injury. Immunologic compromise has no positive affect on translocation, inversely nonspecific immune response has been stimulated in hemorrhagic shock.

**REFERENCES**


