Increased Systemic Inflammation After Laparotomy vs Laparoscopy in an Animal Model of Peritonitis

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Objective: To study the influence of laparotomy and laparoscopy on local and systemic inflammation in a rat model of peritonitis.

Design: Bacteremia, peripheral leukocyte subpopulations, tumor necrosis factor α (TNF-α) plasma levels, and ex vivo secretion of peripheral blood mononuclear cells were investigated after laparotomy and laparoscopy in a prospective randomized experimental study.

Setting: Surgical department of a university hospital.

Animals: 60 male inbred Wistar rats.

Interventions: Standardized fecal inoculum was injected intraperitoneally and rats underwent laparotomy (n=20), laparoscopy (n=20), or no further manipulation (control group, n=20). Blood samples were obtained during the perioperative course to determine bacteremia, leukocytic subpopulations, TNF-α plasma levels, and ex vivo secretion. The number of intraperitoneal abscesses was determined in each animal after 1 week.

Results: One hour after intervention, bacteremia was significantly higher in the laparotomy and laparoscopy groups compared with the control group (P=.01). Fecal inoculum caused significant monocytopenia and lymphocytopenia in all groups within 1 hour after intervention (P<.05), with complete recovery on day 2 only in the laparoscopy and control groups. Laparotomy caused a significant increase in TNF-α plasma levels and decrease of ex vivo production of TNF-α compared with the other 2 groups (P<.05).

Conclusions: Laparotomy and laparoscopy increased the incidence of bacteremia and systemic inflammation in this peritonitis model. The inflammatory response was significantly higher in the laparotomy group compared with the laparoscopy group.

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Laparoscopic surgery is increasingly used for intra-abdominal diseases complicated by inflammatory processes and peritonitis. Successful treatment of appendicitis, perforated peptic ulcer, and diverticulitis has been reported with low morbidity.1-5 However, a theoretical concern is that elevated intra-abdominal pressure may promote bacteremia and systemic inflammatory response during laparoscopic surgery. Data regarding the effects of pneumoperitoneum on physiological changes and systemic inflammation during sepsis are scarce and controversial.5,7 Furthermore, only early effects of pneumoperitoneum during peritonitis have been evaluated and the differences between conventional and laparoscopic surgery have not been compared in all studies.

Therefore, the influence of laparotomy and laparoscopy on bacteremia, peripheral leukocytic subpopulations, proinflammatory tumor necrosis factor α (TNF-α) plasma levels, and ex vivo secretion of peripheral blood mononuclear cells (PBMCs) was studied in a rat model.

RESULTS

MICROBIOLOGICAL ANALYSIS

The number of blood cultures positive for organisms was increased in the laparotomy and laparoscopy group as compared with the control group (P=.01) 1 hour after stool application (Table 1). There was no difference in bacteremia between the groups 1 week after fecal inoculum and surgical intervention.
MATERIALS AND METHODS

EXPERIMENTAL COURSE

Sixty male inbred 3-month-old Wistar rats (Charles River, Sulzfeld, Germany) were acclimated to a climate- and light-cycle–controlled environment for at least 7 days prior to investigations. The animals were allowed standard laboratory food and water ad libitum. All animals were initially anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg) under sterile conditions. A standardized fecal inoculum (1 × 0.5 mL/kg) was applied intraperitoneally under sterile conditions in all animals. The preparation has been previously described and has been shown to produce a nonfatal bacterial peritonitis after intraperitoneal instillation in rats. The rats were randomized into 3 different groups. In the first group (n = 20), laparotomy was performed and the abdomen was closed after 30 minutes. In the second group (n = 20), laparoscopy was performed with insufflation of carbon dioxide at a pressure of 8 mm Hg over a period of 30 minutes. The control group (n = 20) underwent no further manipulation after stool injection. Blood samples were obtained from the femoral artery and placed into sterile heparinized vials (pyrogen-free) 2 days before, 1 hour after, and 2 and 7 days after fecal inoculation. Leukocyte subpopulations were determined with a differential blood smear and plasma was separated from blood samples by centrifugation at 3000g at 4°C for 10 minutes to determine TNF-α plasma levels using commercially available enzyme immunoassays (Laboserv, Gießen, Germany). Additionally, 50 µL of blood from each sample was used to measure the ex vivo TNF-α secretion by PBMCs. Microbiological analysis of the blood was performed before, 1 hour after, and 7 days after intervention. All animals underwent laparotomy on day 7 to determine the number of intraperitoneal abscess formations. All studies were performed under protocols approved by the local committees of animal use and care.

Microbiological analysis of blood cultures detected 12 different bacteria species after laparotomy, 7 species after laparoscopy, and 7 species in the control group (Table 2).

LEUKOCYTE SUBPOPULATIONS

Anesthesia and fecal inoculum caused a significant leukocytopenia 1 hour after intervention in all groups (Figure 1). Leukocyte counts recovered on day 2 in the laparoscopy and control groups (P = .02), while they did not recover in the laparotomy group during the entire postoperative course. In contrast to the laparotomy group, laparoscopy and control groups showed a decrease of leukocyte counts in the late postoperative course (P = .01). Decrease and recovery of the leukocyte count was mainly caused by a change in the number of lymphocytic and monocytic cells (Figure 2 and Figure 3). Decrease of monocytes and lymphocytes was significantly higher after laparotomy compared with laparoscopy and in the control group 1 hour after intervention. Neutrophil granocyte counts increased in the postoperative course (Figure 4). While rats in the laparoscopic and laparotomy groups showed a significant increase 1 hour after fecal inoculum and surgery, fecal inoculum alone caused only a slight increase of neutrophil granulocytes on day 2, with complete recovery after 1 week. Early granulocytosis after laparoscopy or laparotomy was followed by complete recovery on day 2 and a second increase on day 7.

Table 1. Number of Positive Blood Cultures After 1 Hour and 7 Days

<table>
<thead>
<tr>
<th>Time After Intervention</th>
<th>Laparotomy Group (n=20)</th>
<th>Laparoscopy Group (n=20)</th>
<th>Control Group (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>20 (100)</td>
<td>17 (85)</td>
<td>7 (35)*</td>
</tr>
<tr>
<td>7 d</td>
<td>13 (65)</td>
<td>13 (65)</td>
<td>12 (60)</td>
</tr>
</tbody>
</table>

*P < .05, laparotomy and laparoscopy groups vs control group.

TNF-α PLASMA LEVELS AND TNF-α EX VIVO SECRETION BY PBMCs

Plasma was separated from blood samples by centrifugation at 3000g at 4°C for 10 minutes immediately after withdrawal. Tumor necrosis factor α serum levels were determined by using commercially available enzyme immunoassays (Laboserv). Additionally, 50 µL of blood from each sample was diluted with 200 µL of fetal calf serum–supplemented (2% vol/vol) RPMI 1640 (Biochrom, Berlin, Germany) and incubated for 4 hours (37°C, 5% carbon dioxide) with 100 ng/mL of endotoxin Escherichia coli 0127:B8 (Sigma, Deisenhofen, Germany) to assess the ex vivo TNF-α secretion of PBMCs. Cell-free supernatants were harvested by centrifugation at 3000g at 4°C for 10 minutes. All samples were stored in 2-mL pyrogen-free polypropylene screw-cap tubes (Sarstedt, Numbrecht-Rommelsdorf, Germany) at −85°C until final analysis.

MICROBIOLOGICAL ANALYSIS

The microorganisms were grown on chocolate agar (tryptic soy agar supplemented with 10% defibrinated sheep blood, heated to 10 minutes to 80°C), blood agar (Columbia agar supplemented with 5% defibrinated sheep blood), Endo agar, and Sabouraud agar in both an aerobic and anaerobic atmosphere. The notypical identification of all strains was carried out by testing the carbohydrate fermentation reactions or by using commercially available enzyme activity and fermentation tests (API, Bio Merieux, Nuttingen, Germany).

STATISTICS

Data are given as means±SD. Data between groups were compared using the Kruskal-Wallis test for continuous data and the Fisher exact test for categorical data, if appropriate. P < .05 was considered significant.
**TNF-α PLASMA LEVELS AND TNF-α EX VIVO SECRETION BY PBMCs**

Plasma levels of TNF-α sharply increased 1 hour after fecal inoculum and intervention in all groups (Figure 5). Levels were significantly higher after laparotomy compared with laparoscopy and the control group at this time. There were no group differences in the plasma levels of TNF-α on days 2 and 7.

One hour after intervention, the TNF-α ex vivo production of PBMCs decreased significantly in all groups, with significantly lower levels after laparotomy compared with laparoscopy and in the control group (Figure 6). On postoperative days 2 and 7 there was no significant difference among the 3 groups.

**DEVELOPMENT OF INTRAPERITONEAL ABSCESSES**

Intraperitoneal abscess formation was detected in all 20 rats after laparotomy, in 15 of 20 rats after laparoscopy, and in 18 of 20 rats in the control group 1 week after inoculation. The mean number of abscesses in each animal was significantly higher (P<.05) after laparotomy (10±6) compared with laparoscopy (6±5.1) and the control group (5±4.8).

**COMMENT**

Laparoscopic techniques are being increasingly used in surgical conditions complicated by diffuse or localized peritonitis. It has been demonstrated that laparoscopic is superior to conventional open appendectomy in terms of postoperative complications and recovery in prospective randomized trials.1,3,4 Further, laparoscopic management of perforated peptic ulcers has been reported to be simple and followed by a short recovery time.5 In rabbits, Gurtner et al8 did not find an increase of bacteremia or endotoxemia after pneumoperitoneum of 12 mm Hg. In comparison with these results, experimental studies in rats showed a significant increase of extent and severity of peritonitis and bacteremia after laparoscopy and ulcus perforation compared with the control group.9 Unfortunately, a comparison between laparoscopy and laparotomy was not performed in this experiment. In our study, laparoscopy was superior to laparotomy in terms of postoperative systemic inflammation as well as intra-peritoneal abscess formation.

The nonfatal peritonitis produced in the rats resembles the clinical situation and the spectrum of bacterial species (E coli, Enterococcus faecalis, Staphylococcus aureus) found in blood cultures of the rats were similar to what is commonly detected in patients with abdominal sepsis.10,11 The number of blood cultures positive for organisms was significantly higher after laparoscopy as compared with the control group 1 hour after intervention. It has been demonstrated that elevated intra-abdominal pressure leads to an increase of the patency of lymphatic openings through which intra-abdominal fluids are removed from the peritoneal cavity.12 This may explain the difference of bacteremia between these 2 groups. However, aerobic bacteria were not found after 1 hour in the laparoscopy or control groups. Since carbon dioxide is bacteriostatic on aerobic bacteria,13 this may explain why aerobic bacteria were only detected in blood cultures after laparotomy at this time.

Although there was a higher incidence of bacteremia after laparoscopy, systemic inflammation was only slightly increased. While the neutrophil granulocyte count was significantly higher 1 hour after fecal inoculum after laparoscopy compared with the control group, there

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**Table 2. Bacterial Species in Blood Cultures 1 Hour and 7 Days After Endotoxin Application**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>1 h</th>
<th>7 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Laparotomy Group</td>
<td>Laparoscopy Group</td>
</tr>
<tr>
<td>Aerobic gram-negative</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aerobic gram-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus coagulase negative</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Streptococcus viridans</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic gram-negative</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacteroides sp</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacteroides uniformis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic gram-positive</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Prevotella sp</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propionibacterium sp</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bifidobacterium sp</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Plus sign indicates 1 to 3 cultures positive for organisms; + +, 4 to 6 cultures positive; + + +, >6 cultures positive; and ellipses, no organisms found.
was no difference between the other leukocytic subpopulations between these groups during the postoperative course. Plasma levels of TNF-α and ex vivo secretion of PBMCs showed almost parallel changes in the postoperative course, indicating that rats had similar systemic inflammation in both groups. It seems that laparoscopy does not significantly promote systemic inflammation compared with controls, which is also confirmed by the number of intraperitoneal abscess formations showing no difference between the 2 groups.

In contrast to laparoscopy, laparotomy caused a significant increase of systemic inflammation in the
early postoperative course. While leukocyte counts recovered on day 2 in the laparoscopy and control groups, they did not recover after laparotomy and the postoperative decrease of monocytes and lymphocytes was significantly higher after laparotomy. In parallel to the higher incidence of bacteremia, TNF-α plasma levels showed significantly higher values and TNF-α secretion by PBMCs was mostly decreased in the laparotomy group 1 hour after intervention. It may be that abdominal incision causes greater tissue trauma, leading to an increase of proinflammatory cytokines and enhanced postoperative systemic inflammation. In patients undergoing colectomy, systemic inflammation was significantly higher after an open approach than a laparoscopic procedure, which is due to less trauma in the laparoscopic group. This hypothesis is also supported by the histopathological findings in this study. Intraperitoneal abscess formation was detected in all 20 rats after laparotomy and the mean number of abscesses in each animal was significantly higher in this group as compared with the laparoscopy and control groups. Early enhanced postoperative systemic inflammation may cause lower transient immunologic defense after laparotomy, leading to enhanced sepsis in the rats.

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REFERENCES


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