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Does laparoscopy increase bacteremia and endotoxemia in a peritonitis model?

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Abstract
Background: Laparoscopy is increasingly used in patients with intraabdominal bacterial infection although pneumoperitoneum may increase bacteremia by elevated intraabdominal pressure.
Methods: The influence of laparotomy and laparoscopy on bacteremia, endotoxemia, and postoperative abscess formation was investigated in a rat model. Rats received intraperitoneally a standardized fecal inoculum and underwent laparotomy ($n = 20$), or laparoscopy ($n = 20$), or no further manipulation in the control group ($n = 20$).
Results: Bacteremia and endotoxemia were higher after laparotomy and laparoscopy compared to the control group ($p = 0.01$) 1 h after intervention. One hour after intervention, aerobic and anaerobic bacterial species were detected in the laparotomy group while only anaerobic bacteria were found in the other two groups. Although bacteremia and endotoxemia did not differ among the three groups after 1 week, the mean number of intraperitoneal abscesses was significantly higher ($p < 0.05$) after laparotomy ($n = 10$) compared with laparoscopy ($n = 6$) and control group ($n = 5$).
Conclusion: Laparoscopy does not increase bacteremia and intraperitoneal abscess formation compared to laparotomy in an animal model of peritonitis.

Key words: Laparoscopy — Peritonitis — Bacteremia — Endotoxemia

Laparoscopic surgery is currently performed for benign and malignant intraabdominal diseases. Some authors reported successful treatment of inflammatory processes like appendicitis, Crohn's disease, perforated peptic ulcer, or diverticular disease [1, 3, 9, 11, 13]. Furthermore, laparoscopic surgery appears to be feasible in patients with abdominal sepsis without increasing postoperative morbidity and mortality [6]. But there is still some theoretical concern that pneumoperitoneum may cause enhanced bacteremia and endotoxemia due to increased intraperitoneal pressure. Only few and even controversial data exist from experimental studies which have investigated the effects of pneumoperitoneum on bacteremia and physiological changes during peritonitis and sepsis [2, 4, 8]. Furthermore, peritonitis was not caused by different bacterial species but by intraperitoneal inoculation of *Escherichia coli* alone in all studies.

Thus, it remains questionable whether laparoscopic surgery may be harmful in patients with diffuse peritonitis. Since there are to our knowledge no studies which have compared the influence of surgical intervention on early and late outcome in peritonitis, the influence of laparotomy and laparoscopy on bacteremia, endotoxemia and postoperative intra- and extraperitoneal abscess formation was investigated in a peritonitis model.

Materials and methods

A standardized fecal inoculum of human stool was chosen for this study because it produces a nonfatal bacteremia after intraperitoneal instillation in rats [10]. It further has been demonstrated that inoculation of heat-inactivated stool suspension did not cause intraabdominal peritonitis in this model. Therefore peritonitis was caused by bacterial infection and not by toxic noninflammatory effects of the stool suspension.

After stool injection, rats were randomized into three groups (group I: laparotomy; group II: laparoscopy; group III: control group). The hypothesis of the experiment was that laparoscopy with carbon dioxide leads to enhanced bacteremia, endotoxemia, and development of intraperitoneal abscess formation. The endpoints of the study were perioperative changes in bacteremia and endotoxemia and the incidence and number of intraperitoneal abscess formation 1 week after intervention.

Animals

Sixty male inbred 2-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 studies were performed under protocols approved by the local committees of Animal Use and Care.

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Experimental course and operative procedures

All animals were anesthetized by intraperitoneal injection of pentobarbital 60 mg/kg under sterile conditions. It was verified by a chromogenic *Limulus* amoebocyte lysate assay (Whittaker Bioproducts, Wakersville, Maryland, USA) that pentobarbital did not contain endotoxins. Stool suspension (1 × 0.5 ml/kg) was then intraperitoneally applied transcutaneously under sterile conditions in all animals. The rats were randomized into three different groups. A 10-cm midline laparotomy was accomplished in the first group ($n = 20$) and the abdomen was closed after 30 min. In the second group, pneumoperitoneum was performed through a Veress needle with insufflation of carbon dioxide ($n = 20$) at a pressure of 10 mmHg over 30 min. The control group ($n = 20$) underwent no further manipulation after stool injection. Blood samples were taken from the femoral vein of the rats and placed into sterile heparinized vials (pyrogen-free) before, 1 h, 2 and 7 days after surgery to determine endotoxin levels of the blood by using a chromogenic *Limulus* amoebocyte lysate assay. Microbiologic phenotypical identification of bacteria in the blood cultures was performed before, 1 h, and 1 week after stool application. The animal underwent laparotomy on postoperative day 7 to determine the number and location of intraperitoneal abscess formations. Abscesses were excised, analyzed microbiologically, and confirmed histologically using HE stains. Additionally, microbiologic analysis of peritoneal swabs was performed to evaluate the differences between intraperitoneal and intravenous bacterial species on day 7.

Microbiological analysis

The microorganisms were grown on chocolate agar (Tryptic Soy agar supplement with 10% defibrinated sheep blood, heated for 10 min to 80°C), blood agar (Columbia agar supplemented with 5% defibrinated sheep blood), Endo agar, and Sabouraud agar in an aerobic and anaerobic atmosphere. The phenotypical identification of all strains was verified by testing the carbohydrate fermentation reactions or by using commercially available enzyme activity and fermentation tests (API, Bio Mérieux, Nürtingen, Germany).

Measurement of endotoxin blood levels

Plasma was separated from blood samples by centrifugation at 3,000*g* at 4°C for 10 min. Plasma samples were diluted tenfold and heated to 70°C for 5 min to eliminate endotoxin inhibitors. All samples were stored in 2-ml pyrogen-free polypropylene screw cap tubes (Sarstedt, Numbrecht-Rommelsdorf, Germany) at −85°C until final analysis.

A chromogenic *Limulus* amoebocyte lysate (LAL) assay (Whittaker Bioproducts, Walkersville, Maryland, USA) was used to determine endotoxin serum levels; 50 μl of each plasma sample and 50 μl of LAL were pipeted into wells of a sterile microtiter plate (Falcon No. 3072, Becton Dickinson, Lincoln Park, New Jersey, USA). After incubation at 37°C for 10 min, 100 μl of chromogenic substrate (Acetyl-Ile-Glu-Gly-Arg-p-nitroanilide, 1.6 mmol/l) was added. The reaction was stopped by 100 μl of 25 % (v/v) acetic acid after 6 min and absorbance of each well was measured at 405 nm with a microplate reader.

Endotoxin standards at concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 U/ml were prepared from *E. coli* 0111:B4 (Whittaker Bioproducts) and two blanks were performed in parallel with each test. In this assay, 12 endotoxin units correspond to 1 ng of *E. coli* 0111:B4 lipopolysaccharide (LPS).

Statistics

Data are given as mean and standard deviation. Data between groups were compared using a Kruskal-Wallis test for continuous data and Fisher's exact test for categorical data, if appropriate. *P* values less than 0.05 were considered significant.

Results

Bacteremia was not found in any animal at the beginning of the experiment. One hour after stool application, incidence

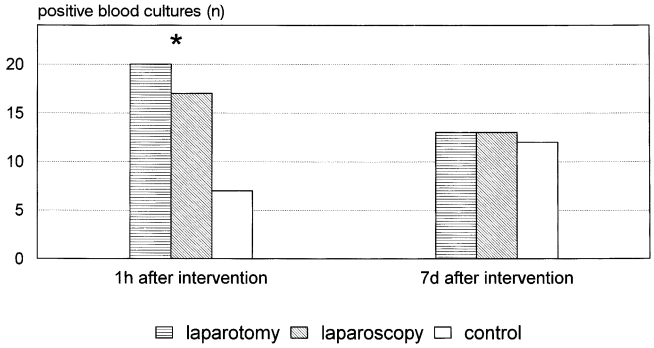

Fig. 1. Incidence of positive blood cultures after laparotomy ($n = 20$), laparoscopy ($n = 20$), and control group ($n = 20$).

of bacteremia increased after laparotomy and laparoscopy compared to the control group ($p < 0.001$) (Fig. 1). There was no statistical difference in bacteremia between all groups 1 week after fecal inoculum and surgical intervention. Positive peritoneal cultures did not significantly differ between laparotomy (20/20), laparoscopy (15/20), and control group (18/20) at the end of the experiment.

Qualitative microbiologic analyses have detected 42 different bacteria in assays of the human stool inoculum. Analysis of the stool taken from the intestinum of the rats have found 35 different bacterial organisms.

Sixteen different species out of human fecal inoculum and rat stool species were detected in blood culture and peritoneal swabs with different distribution between the three groups (Table 1). Five of these bacteria were not found in fecal inoculum but in stool of the rats. While rats in the laparotomy group showed both anaerobic and aerobic bacteria in blood cultures, aerobic bacteria were not detected in blood cultures after laparoscopy and in the control group 1 h after intervention. Anaerobic *Bifidobacterium* spp. and *Bifidobacterium adolescentis* were most often found in blood culture 1 h after intervention in all groups. Although the number of positive blood cultures decreased on postoperative day 7, various anearobic and aerobic bacteria were detected in all groups.

Microbiologic analysis of peritoneal fluid detected 12 different organisms after laparotomy, nine species after laparoscopy, and only eight species in the control group 1 week after stool inoculation. *E. coli* and *Enterococci* were the most frequent species found intraperitoneally in all groups. These species showed only in part coincidence with organisms detected in blood cultures on postoperative day 7.

No animal had endotoxemia at the beginning of the experiment. Plasma endotoxin levels increased within 1 h after stool inoculum in all groups ($p < 0.01$) (Fig. 2). Levels were higher ($p < 0.01$) in animals after laparotomy compared with laparoscopy and control group 1 h after intervention but were not different during the following postoperative course. Endotoxin levels already had reached their maximum peak 1 h after intervention in all groups followed by a slight decrease toward the end of the observation on day 7.

No animal died before scheduled autopsy 1 week after stool inoculation. Iatrogenic injury from insertion of the Veress needle or from abdominal incisions did not occur in any animal. Intraperitoneal abscess formation was con-

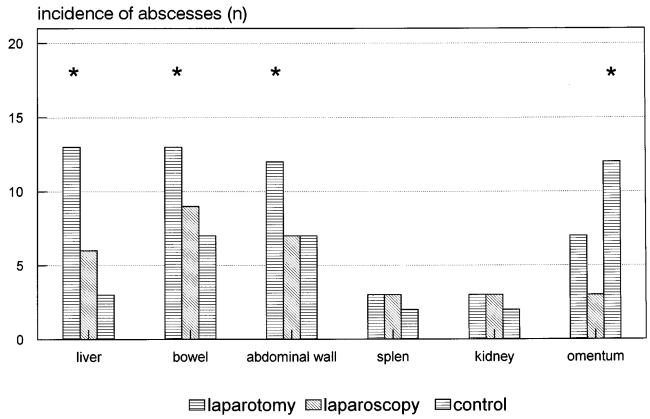

Fig. 2. Endotoxin level in blood after laparotomy ($n = 20$), laparoscopy ($n = 20$), and control group ($n = 20$) (mean and standard deviation, asterisk indicates $p < 0.01$: laparotomy vs laparoscopy and control group).

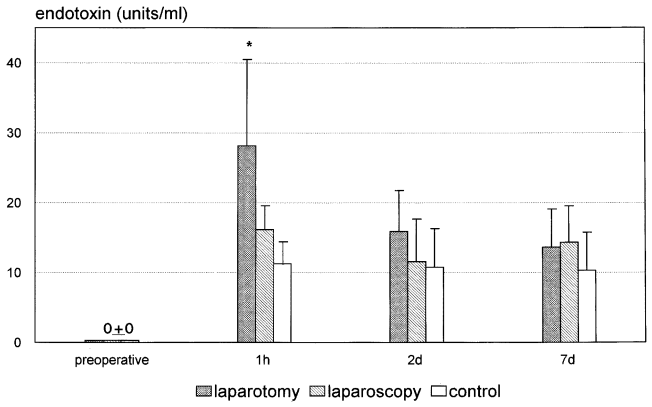

Fig. 3. Incidence and organ distribution of intraperitoneal abscess formations after laparotomy ($n = 20$), laparoscopy ($n = 20$), and control group ($n = 20$) (asterisk indicates $p < 0.05$: laparotomy vs laparoscopy vs control group).

Table 1. Bacterial species in blood cultures and peritoneal fluids[a]

Bacteria	Human feca inoculum	Rat intestinum	Blood culture 1 hour			Blood culture 7 days			Intraperitoneal		
			Laparotomy	Laparoscopy	Control	Laparotomy	Laparoscopy	Control	Laparotomy	Laparoscopy	Control
Aerob gram-negative											
Escherichia coli	■	■	+			+		+	+++	+++	+++
Proteus mirabilis		○				+			++	++	+
Aerob gram-positive											
Enterococcus faecalis	■	■	++			+			+++	+++	+++
Bacillus spp.	□		+			+			+		
Staphylococcus aureus	■	■				+	+		+	+++	+
Staphylococcus coag. neg.		○	+								
Streptococcus viridans		○						+	+	+	+
Anaerob gram-negative											
Bacteroides spp.	■	■		+			+		++		++
Bacteroides fragilis	■	■				+	+	+	++	+	++
Bacteroides uniformis	■	■	+	+	+						
Bacteroides ovatus		○							+	+	++
Anaerob gram-positive											
Prevotella spp.	■	■				+			+	+	
Clostridium perfringens	□								+	+	
Propionibacterium spp.	■	■	+			+	+	+	+		
Bifidobacterium spp.		○	+++	+++	++	+	+				
Bifidobacterium adolescentis	□		+++	++	++						

[a] *Key to symbols:* ■: detected in human fecal inoculum and rat intestinum; □: only detected in fecal inoculum; ○: only detected in rat intestinum; +: 1–3 positive cultures; ++: 4–6 positive cultures; +++: >6 positive cultures.

firmed in all 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 10 ± 6.2 after laparotomy, 6 ± 5.1 after laparoscopy, and 5 ± 4.8 in the control group and differed ($p < 0.05$) between the first and the two other groups.

The localization of intraabdominal abscesses also differed ($p < 0.01$) between the groups (Fig. 3). In the laparotomy group, abscesses were most often localized at the peritoneal surface of the liver, on the bowel surface and the abdominal wall. Interenteric abscesses were only found in five rats after laparotomy and did not occur in the two other groups. In laparoscopic group, abscesses were increasingly found at the peritoneal surface of the bowel and the abdominal wall while in the control group abscesses were most often found in the omentum majus.

Discussion

Laparoscopic techniques are sometimes utilized in patients with diffuse or localized peritonitis. It has been demonstrated in prospective randomized trials that laparoscopic appendectomy is superior or at least does not differ compared to open appendectomy in terms of postoperative complications, hospital stay, and recovery [1, 11]. Urbano et al. further demonstrated that laparoscopic surgery of perforated peptic ulcers is simple, rapid, and followed by a quick recovery [13]. In contrast to these results, Eypasch et al. reported higher morbidity after laparoscopic treatment of perforated peptic ulcer than after a conventional approach [5]. Although the reported results may also be influenced by selection of patients and experience in laparoscopic techniques, it is hypothetical that continuous elevated intraab-

dominal pressure promotes bacterial translocation and thus increases postoperative septic complications. This is supported by experimental studies which showed an increase in the extent and severity of peritonitis as well as bacteremia after perforated peptic ulcer and laparoscopy compared to the control group in a rat model [2]. However, the data of experimental studies regarding laparoscopic surgery during peritonitis remain controversial. Eleftheriadis et al. [4] have demonstrated that elevated intraabdominal pressure (15 mmHg) leads to intestinal ischemia, to oxygen free-radical production, and to increased bacterial translocation in rats, while Gurtner et al. [8] did not find an increase of bacteremia or endotoxemia after pneumoperitoneum of 12 mmHg in rabbits. Unfortunately, laparotomy and laparoscopy were not compared in these studies.

In the present study, a nonfatal peritonitis was induced, stimulating the clinical stiuation with intraabdominal pus, abscess formations, and positive blood cultures of different bacterial species. The spectrum of bacterial species (*Escherichia coli, Enterococcus faecalis, Staphylococcus aureus*) found in blood cultures and peritoneal fluids of the rats is similar to the spectrum commonly detected in patients with abdominal sepsis [15, 16].

Overall, 16 different bacteria were found in blood cultures and peritoneal fluid of all animals. Five of the detected bacteria were not found in fecal inoculum, which may indicate that these germs are probably translocated from the rat intestinum during the development of peritonitis.

The incidence of positive blood cultures was significantly higher after laparoscopy compared to control group 1 h after intervention but showed no difference after 7 days. It is well known that intraabdominal fluids, cells, and particles are removed from the peritoneal cavity via large terminal lymphatics connected to the thoracic duct and venous system. The openings of these lymphatics are located on the peritoneal surface of the diaphragm between the lateral borders of peritoneal mesothelial cells [14]. It has been demonstrated that elevated intraabdominal pressure leads to an increase of the patency of these lymphatic openings in laparoscopy [12], which may explain the difference in bacteremia between the laparoscopic and control group. However, laparotomy promoted bacterial translocation and bacteremia even more. Blood cultures of rats detected more different bacterial species after laparotomy than after laparoscopy. Furthermore, blood cultures of the rats showed both anaerobic and aerobic bacteria after laparotomy while aerobic bacteria were only detected once in blood cultures after laparoscopy. This is confirmed by higher plasma endotoxin levels after laparotomy compared to laparoscopy and the control group 1 h after intervention. Since carbon dioxide is bacteriostatic on aerobic bacteria [7] this may explain why aerobic bacteria were only detected in blood cultures of one animal after laparoscopy. Interestingly, there was no statistical difference between all groups in bacteremia and endotoxemia after 1 week. The early translocation may be promoted by the different operations while late translocation is caused by the development of peritonitis. The difference in early endotoxemia between rats underling laparotomy and those in either the laparoscopy or control group may be caused by a transient bacteremia of specific aerobic organisms. It also may be that the incision of the abdominal wall with injury of blood vessels caused higher translocation of bacteria in the laparotomy group. The enhanced perioperative strain during laparotomy is confirmed by the significantly higher number of intraperitoneal abscess formations in this group.

Nevertheless, perioperative treatment with antibiotic may reduce the differences between the groups. Therefore, further experimental and clinical trials are needed to analyse different adjuvant therapeutic strategies in this model.

In summary, laparoscopy does initially increase the incidence of positive blood cultures compared to control group in an animal model of peritonitis. But endotoxemia and the development of intraabdominal abscesses are not increased by laparoscopy compared with the control group. In contrast to this, laparotomy significantly promotes transient translocation of aerobic and anaerobic species, endotoxemia, and development of intraperitoneal abscesses compared to laparoscopy and the control group.

References

1. Attwood SE, Hill AD, Murphy PG, Thorton J, Stephens RB (1992) A prospective randomized trial of laparoscopic versus open appendectomy. Surgery 112: 497–501
2. Blöchle C, Emmermann A, Achilles E, Treu H, Zornig C, Broelsch CE (1995) Einfluß des Penumoperitoneums auf Ausdehnung und Schwere einer durch peptische Ulkusperforation induzierten Peritonitis der Ratte. Langenbecks Arch Suppl: 1–5
3. Böhm B, Schwenk W, Müller JM (1996) Laparoskopische kolorektale Chirurgie. Dtsch Med Wochenschr (submitted)
4. Eleftheriadis E, Kotzampassi K, Papanotas K, Heliadis N, Sarris K (1996) Gut ischemia, oxidative stress, and bacterial translocation in elevated abdominal pressure in rats. World J Surg 20: 11–16
5. Eypasch E, Menningen R, Paul A, Troidl H (1993) Die Bedeutung der Laparoskopie bei der Diagnostik und Therapie des akuten Abdomens. Zentralbl Chir 118: 726–732
6. Geis WP, Kim HC (1995) Use of laparoscopy in the diagnosis and treatment of patients with surgical abdominal sepsis. Surg Endosc 9: 178–182
7. Gill CO, Delacy KM (1991) Growth of Escherishia coli and Salmonella typhimurium on high > pH beef packed under vacuum or carbon dioxide. Int J Food Micribiol 13: 21–30
8. Gurtner GC, Robertson CS, Chung SCS, Ling TKW, IP SM, Li AKC (1995) Effect of carbon dioxide pneumoperitoneum on bacteraemia and endotoxaemia in an animal model of peritonitis. Br J Surg 82: 844–848
9. Hansen JB, Smithers BM, Schache D, Wall DR, Miller BJ, Menzies BL (1996) Laparoscopic versus open appendectomy: A prospective randomized trial. World J Surg 20: 17–21
10. Lorenz W, Reimund KP, Weitzel F, Celik I, Kurnatowski M, Schneider C, Mannheim W, Heiske A, Neumann K, Sitter H, Rothmund M (1994) Granulocyte colony-stimulating factor prophylaxis before operation protects against lethal consequences of postoperative peritonitis. Surgery 116:925–934
11. Tate J, Dawson JW, Chung SC, Lau WY, LI AK (1993) Laparoscopic versus open appendectomy: prospective randomised trial. Lancet 342: 633–637
12. Tsilibary EC, Wissig SL (1983) Lymphatic absorption from the peritoneal cavity: regulation of patency of mesothelial stomata. Microvasc Res 25: 22–39
13. Urbano D, Rossi M, De Simone P, Berloco P, Alfani D, Cortesini R (1994) Alternative laparoscopic management of perforated peptic ulcers. Surg Endosc 8: 1208
14. Walker AP, Condon RE (1989) Peritonitis and intraabdominal abscesses. Principles of surgery. 5th ed. Mc Graw-Hill, New York, pp 1459–1489
15. Walker AP, Krepel CJ, Gohr CM, Edmiston CE (1994) Microflora of abdominal sepsis by locus of infection. J Clin Microbiol 32: 557–558
16. Zubkov MN, Menshikov DD, Gugutsidze EN, Chegin VM, Vasina TA (1995) Microbiologic diagnosis of mixed anaerobic and aerobic surgical infections. Antibiot Khimioter 40: 46–50