The influence of omentectomy on bacterial clearance: an experimental study

Omentektominin bakteriyel çoğalma üzerine etkisi: Deneysel çalışma

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BACKGROUND

The influence of an omentectomy on peritoneal defense mechanisms and its clinical consequences have not been fully elucidated. In the present study, we aimed to investigate the influence of omentectomy on bacterial growth in blood and tissue specimen cultures in rats with experimental peritonitis.

METHODS

Fifty Wistar-Albino rats were included in the present study. Animals were assigned into five groups as follow: Group 1 (n=10), omentectomy alone; Group 2 (n=10), omentectomy + bacterial peritonitis; Group 3 (n=10), bacterial peritonitis alone; Group 4 (n=10), laparotomy alone; and Group 5 (n=10), sham group. Culture positivity rate and bacterial growth (colony forming units [CFU]/gram tissue) were assessed in mesenteric lymphoid tissue and venous blood of all animals.

RESULTS

Bacterial growth in lymphoid tissue was significantly higher in Groups 2 and 3 than others (both, $p<0.05$). CFU of *Escherichia coli* in lymphoid tissue was significantly higher in Group 2 than in Groups 1 and 3 (both, $p<0.05/4$). Blood culture positivity was significantly higher in Group 2 than the others (both, $p<0.05$).

CONCLUSION

The omentum has an important role in the host peritoneal defense system. Peritoneal infection may pursue a more severe course with increased bacterial entrance into the blood in the absence of the omentum.

Key Words: Bacterial growth; experimental study; peritonitis; peritoneal defense; rats, Wistar Albino; omentectomy.

AMAÇ

Omentektominin periton defans mekanizmaları üzerine etkisi ve bunun klinik sonuçları her yönü ile incelenmiş değildir. Bu çalışmada, deneysel peritonit oluşturulan sıçanlarda omentektominin kan ve doku örneklerindeki bakteriyel çoğalma üzerine etkisini araştırmayı amaçladık.

GEREÇ VE YÖNTEM

Çalışmaya 50 adet Wistar Albino cinsi sıçan dahil edildi. Denekler 5 gruba ayrıldı: Grup 1 (n=10), yalnızca omentektomi; grup 2 (n=10), omentektomi+bakteriyel peritonit; grup 3 (n=10), yalnızca bakteriyel peritonit; grup 4 (n=10), yalnızca laparotomi; grup 5 (n=10), sham grubu. Tüm deneklerin mezenterik lenfoid doku ve venöz kanlarında kültür pozitifliği oranı ve üreyen bakteri koloni sayısı değerlendirildi.

BULGULAR

Grup 2 ve grup 3'teki bakteriyel çoğalma oranı diğer gruplardan anlamlı olarak daha yüksek idi (tümü için, $p<0,05$). Lenfoid dokuda üreyen *Escherichia coli* koloni sayısı grup 1 ve grup 3'ten anlamlı olarak daha yüksek idi (her ikisi için, $p<0,05$). Kan kültürü pozitifliği grup 2'de diğer gruplardan anlamlı olarak daha yüksek idi (tümü için, $p<0,05$).

SONUÇ

Omentum, periton defans sistemininde önemli bir role sahiptir. Omentumun olmaması halinde bakterilerin kana karışması artacağından peritonda enfeksiyon daha şiddetli bir seyir izleyebilir.

Anahtar Sözcükler: Bakteriyel çoğalma; deneysel çalışma; periton savunması; peritonit; sıçan, Wistar Albino cinsi; omentektomi.

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Bacterial peritonitis is a serious clinical condition with high mortality rate.[1] In addition to the number of bacteria reaching the peritoneum, local and systemic defense mechanisms of the host play a role in the development of bacterial peritonitis. The bacteria reaching the peritoneal space first encounters the local peritoneal defense mechanism such as removal of the bacteria from the peritoneal space by trans-lymphatic absorption, lysis of the bacteria in the peritoneal space by the complement system and phagocytosis by neutrophils and macrophages, and prevention of bacterial invasion into the systemic circulation by fibrin trapping and omentum.[2,3]

Omentectomy is a part of certain surgical procedures. The influence of the removal of the omentum on peritoneal defense mechanisms and its clinical consequences have not been fully elucidated yet. In contrast to studies suggesting that the omentum has a major role in the peritoneal defense mechanisms,[4-8] some authors have asserted that the influence of an omentectomy on the peritoneal defense mechanisms has been overstated[9] and that the omentum might be a source of bowel obstruction due to implantation and growth of tumor cells.[10]

In spite of the majority of the reports concerning the omental macrophage content and the role of the omentum in intraperitoneal phagocytosis,[4-7,9,10] there is no study in the literature investigating the influence of omentectomy on the severity of bacteremia and the number of bacteria growing in the blood and tissue specimen cultures following intraperitoneal injection of bacteria.

Thus, in the present study, we aimed to investigate the influence of omentectomy on the bacterial growth in the blood and tissue specimen cultures (colony forming units per gram of tissue [CFU/g]) in rats with experimental peritonitis.

MATERIALS AND METHODS

Animals

Fifty 12-week-old Wistar-Albino female rats weighing 275±25 g were obtained and acclimatized for 10 days before the study. All rats were in good health and kept under the same physical and environmental conditions in our experiment laboratory. Rats were fed with standard pellet diet and tap water during the study period. Rats were kept in the room with a temperature of 23±2ºC maintained on a 12-hour light/dark cycle. All rats received humane care and the study protocol was carried out in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the local institutional review committee.

The rats were randomly assigned into five groups as: Group 1 (n=10), omentectomy; Group 2 (n=10), omentectomy plus bacterial peritonitis; Group 3 (n=10), bacterial peritonitis without omentectomy; Group 4 (n=10), laparotomy without omentectomy and bacterial peritonitis; and Group 5 (n=10), sham group.

Surgical Procedure

Except in Group 5, all groups underwent laparotomy. The operative procedures were done under sterile condition. Laparotomy was performed through a 2-cm midline incision following ether anesthesia. In Groups 1 and 2, the omentum was removed by meticulous dissection and electrocoagulation of gastroepiploic vessels. Additionally, in Group 2, 1 cc (10^6/ml) *Escherichia coli* (*E. coli*) (ATCC 25922) was injected into the peritoneal cavity. The last procedure was also applied to Group 3 without omentectomy. After hemostasis, the abdominal wall was closed with 4/0 polyglactin 910 (Vicryl®, Ethicon, Somerville, NJ, USA) in two layers. No procedure was performed in rats of Group 5 and they were maintained under the same conditions during the study.

All animals, including those in Group 5, were sacrificed with ether overdose at the 72nd hour of operation. Laparotomy procedure was repeated in all sacrificed rats. After laparotomy, specimens were obtained for microbiological studies from all animals as follows: 5-6 ml venous blood was drawn from the vena cava inferior using 10 ml injector with 20G needle; paraaortic and mesenteric lymph nodes were resected from all sacrificed rats.

Microbiological Studies

The lymphoid tissues were homogenized with a sterile blender and cultured as a 10- fold dilution series for quantitative analysis. For this purpose, 100 µL of each dilution was cultured on Blood Agar Base (Merc, Germany) and EMB agar (Merc, Germany). After 48 h incubation at 37°C under aerobic conditions, the microorganisms were identified using standard microbiological techniques. Bacterial growth was expressed as 10 log number colony forming units per gram of tissue (CFU/g).

All blood samples were cultured aerobically using the BacTec Ped (Becton Dickinson, Diagnostic Instrument Systems, Sparks, MD, USA). Blood cul-

tures were continuously monitored for seven days. Positive cultures were plated out on appropriate media and species identified by sceptor microdilution and standard bacteriological techniques.

Statistical Analysis

Data were reported as median and range. Continuous variables were compared using Mann-Whitney U or Kruskal-Wallis H test. Chi-square test was used to compare nominal variables of culture positivity rate of each group. The statistical significance was defined as a $p<0.05/3$ for Kruskal-Wallis H test, otherwise $p<0.05$. Statistical analyses were performed using the SPSS 11.0 program for Windows.

RESULTS

Lymphoid Tissue

Lymphoid tissue specimen culture positivity rates of Groups 1, 2, 3 and 4 were 50%, 100%, 100% and 10%, respectively. Culture positivity rates in Groups 2 and 3 were significantly higher than in Groups 1 and 4 (both, $p<0.05$). Similarly, Group 1 had a higher culture positivity rate than Group 4 ($p<0.05$). No bacterial growth was observed in the lymphoid tissue specimen culture of Group 5. *E. coli* was isolated in all lymphoid tissue specimen cultures in Groups 2 and 3, but in only 40% of Group 1. *E. coli* was not isolated in the lymphoid tissue specimen culture of Group 4. The other bacteria isolated in lymphoid tissue cultures were as follow:

- *Proteus mirabilis*, in 2 rats of Group 1, in 2 rats of Group 2 (in addition to *E. coli*) and in 1 rat of Group 3.

- *Klebsiella pneumoniae*, in 1 rat of Group 1 and in 2 rats of Group 2 (in addition to *E. coli*).

- *Pseudomonas aeruginosa*, in 1 rat of Group 4.

The median colony numbers of *E. coli* grown in lymphoid tissue specimen cultures of all groups are shown in Table 1. The colony number of *E. coli* growing in lymphoid tissue specimen was found to be significantly higher in Group 2 than Groups 1 and 3 (both, $p<0.05/3$). Group 3 had a higher number of E. coli growing in lymphoid tissue specimens than Group 1 ($p<0.05/3$).

Table 1. *E. coli* growth (colony number) in lymphoid tissue specimen cultures

Groups	Colony number (CFU $\log_{10}$/g) median (range)
Group 1	3.32 (3.08- 3.54)
Group 2	7.7 (6.53- 8.5)
Group 3	6.40 (5.87- 6.8)
Group 4	–
Group 5	–

Group 1, omentectomy alone; Group 2, omentectomy plus experimental bacterial peritonitis; Group 3, experimental bacterial peritonitis alone; Group 4, laparotomy alone; Group 5, sham group.
CFU/g: Colony forming units per gram of tissue.

Blood Culture

Blood culture positivity rates of Groups 1, 2 and 3 were 30%, 80% and 40%, respectively. Culture positivity rate in Group 2 was significantly higher than in Groups 1 and 3 (both, $p<0.05$). No statistically significant difference was observed between Groups 1 and 4 in culture positivity rate ($p>0.05$). No bacterial growth was observed in blood cultures of Groups 4 and 5. *E. coli* was isolated from all blood cultures of Groups 2 and 3. The bacteria isolated in the blood cultures of Group 1 were as follows: *E. coli* in 2 rats and *Proteus mirabilis* in 1 rat.

DISCUSSION

Macrophages are the main component of the intraperitoneal defense system of the peritoneum. In the presence of intraperitoneal infection, they become active in the phagocytosis of the bacteria.[4] Monocytes, granulocytes, histiocytes and mast cells located in the omentum are the other components of the peritoneal defense system. There are conflicting ideas about the sources of the peritoneal macrophages. One of the accepted concepts is that these cells mainly originate from bone marrow and eventually enter the peritoneal spaces.[11] Another concept is that these cells originate from milky spots.[12] Milky spots, which are found on the omental mesothelium, have an important role in the peritoneal defense system.[7] The production of macrophages by the omentum has been shown *in vivo*.[13] It has also been shown that the macrophages were the most abundant phagocytic cells in the milky spots,[14] and that the macrophages originating from milky spots play an important role in the elimination of bacteria.[7]

In one study of Agalar et al.,[15] it was shown that omentectomy might cause important changes in total peritoneal cell count and types, and the macrophages are the most affected cell type in the peritoneum. They reported that chemotaxis of neutrophils in the peritoneal fluid is adversely influenced by omentectomy in either the early or late period of operation. They found that impaired chemotaxis in the early period improved after 30 days. They suggested that the reduction in chemotaxis might be a result of the alteration of a regulatory effect of macrophages on

polymorphonuclear leukocyte chemotaxis caused by qualitative and quantitative changes in peritoneal macrophages or the surgical trauma of omentectomy. Ratajczak et al.[16] reported that stimulation of macrophage colonies was one of the functions of the omentum. Additionally, colony-stimulating factor 1 secreted by omental stromal cells was shown to be necessary for the production and development of peritoneal macrophages.[7,16] It has also been shown that survival after peritonitis in rats with omentectomy was less than in rats without omentectomy.[17]

In contrast to the reports mentioned above, in one study of Agca et al.,[9] it was reported that omentectomy had no adverse effect on peritoneal bactericidal activity. They stated that decrease in the defensive activity of the peritoneum might be supplemented by the systemic macrophages.

Although the omental macrophage content and the role of the omentum in intraperitoneal phagocytosis have been investigated in several studies,[4,7-10] the influence of omentectomy on the severity of bacteremia and the number of bacteria growing in the blood and tissue specimen cultures was not previously investigated.

In the present study, we observed that the number of bacteria growing in lymphoid tissue specimen cultures was higher in rats with omentectomy plus experimental bacterial peritonitis than in omentectomy alone and bacterial peritonitis alone groups. In concordance with the literature,[15,17] it can be suggested that the excess in the number of bacteria growing in lymphoid tissue specimen cultures of the rats with omentectomy plus experimental bacterial peritonitis might be a result of insufficient bacterial clearance by peritoneal macrophages of rats who underwent omentectomy. In addition, the bacterial growth in lymphoid tissue specimen cultures of the omentectomy alone group that developed in the absence of intraperitoneal bacteria injection is the other important finding that supports the role of the omentum in the peritoneal defense mechanism.

Additionally, we observed that rats with omentectomy plus experimental peritonitis had a higher blood culture positivity rate than rats with experimental peritonitis alone. This finding is also suggestive of the role of the omentum in the clearance of bacteria. No significant difference was observed in the blood culture positivity rate of rats with omentectomy alone and those with experimental peritonitis alone. Nevertheless, the blood culture positivity in the absence of intraperitoneal bacteria injection that was observed in rats with omentectomy alone in conjunction with no bacterial growth in the blood culture of rats with laparotomy alone and the sham group are the other important findings that support the role of the omentum in bacterial clearance.

In conclusion, the omentum has an important role in the host peritoneal defense system. Peritoneal infection may pursue a more severe course in the absence of the omentum. Thus, the omentum should be preserved in certain surgical procedures to the extent possible.

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