Original Paper

European Surgical Research

Eur Surg Res 2003;35:35–40
DOI: 10.1159/000067033

Received: December 6, 2001
Accepted after revision: April 30, 2002

Influence of Omentectomy on Peritoneal Defense Mechanisms in an Experimental Model of Intra-Abdominal Infection

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Key Words
Omentectomy · Peritoneal defense mechanisms · Intra-abdominal sepsis · Antibacterial activity, peritoneal fluid

Abstract

Background/Aim: The omentum has an important role as part of peritoneal defense mechanisms. The aim of this study is to show the bactericidal activity of peritoneal fluid and the role of the omentum as a peritoneal defense mechanism in experimental animals with intra-abdominal infections. ***Methods:*** 40 male Spraque-Dawley rats weighing between 250 and 300 g were used in this study. The rats were randomly divided into four groups consisting of 10 animals. The operative procedures were done under sterile conditions. In group I sham laparotomy was done. In group II, the distal part of the cecum was ligated, and cecum perforation was performed. In group III, total omentectomy was performed after cecal ligation and perforation. In group IV only omentectomy was performed. Baseline and 2- and 4-hour peritoneal fluid samples were taken using a Pasteur pipette during laparotomy under anesthesia. Total peritoneal cells counts, bactericidal activity of peritoneal fluid, and types of phagocytic cells in the peritoneal fluid were assessed. ***Results:*** As compared with baseline values, the total peritoneal cell counts were increased at the 2nd and 4th h in all groups ($p < 0.05$). A significant increase was observed after 4 h as compared with 2 h in sham laparotomy, cecal ligation + perforation + omentectomy, and omentectomy groups ($p < 0.05$). A significant increase in the cell counts after 2 h was found in the other groups when compared to the sham laparotomy group ($p = 0.0001$). After 4 h, there was a significant difference between the groups, but especially prominent in the cecum ligation + perforation + omentectomy group ($p = 0.0001$). Proliferating colony counts of *Escherichia coli* and *Pseudomonas aeruginosa* decreased after 2 h, and there was no proliferation in the subsequent cultures. It was observed that the macrophage counts significantly increased after 2 and 4 h as compared with baseline in intragroup assessments ($p = 0.0001$). In the intergroup assessment, an increase was observed in the macrophage counts at baseline and after 2 and 4 h, and this was significant in the cecal ligation + perforation + omentectomy group ($p = 0.0001$). In the omentectomy group, a significant decrease was observed in the macrophage counts between the 2nd and

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0014–312X/03/0351–0035$19.50/0

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4th h (p = 0.0001). ***Conclusion:*** Removal of the omentum in the presence of intra-abdominal infections causes the peripherally derived macrophages to take over the defensive role of macrophages of peritoneal origin as a compensatory mechanism, thus the peritoneal bactericidal activity against *E. coli*, the major pathogen in intra-abdominal infections, does not change after omentectomy.

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Introduction

The peritoneal defense mechanisms are defined as removal of bacteria from the peritoneal space by translymphatic absorption, lysis of bacteria in the peritoneal space by the complement system and phagocytosis by neutrophils and macrophages, and prevention of invasion of bacteria into the systemic circulation by fibrin trapping and omentum [1–3]. Stimulation of the peritoneum by chemical agents, bacteria, or bacterial toxin activates the peritoneal defense mechanisms. Special pores in the mesothelium of the omentum called milky spots are considered as a lymphoid organ [4]. The functions of the milky spots are phagocytosis, antibody production, filtration of the abdominal cavity, trapping tumoral cells, and selective storage of foreign substances like bacteria [5]. The aim of this study was to elucidate the bactericidal activity of peritoneal fluid and the role of the omentum as a peritoneal defense mechanism in experimental animals with intra-abdominal infections.

Materials and Methods

Animals

40 male Spraque-Dawley rats weighing between 250 and 300 g were used. The animals were supplied by the Laboratory of Microbiology of the Cerrahpaşa Medical Faculty of the Istanbul University. The rats were fed with a standard laboratory diet and water ad libitum.

Study Design

The rats were randomly divided into four groups consisting of 10 animals. 25 mg/kg ketamine HCl was injected intramusculary for anesthesia. The abdominal wall was shaved and cleansed with Betadine solution. Surgery was done under sterile conditions. The abdominal cavity was opened by a median incision 3 cm in length. In all groups, 1 ml of sterile saline was administered into the peritoneal cavity by a Pasteur pipette, and then fluid of the peritoneal cavity was withdrawn with the same pipette, so baseline (0 h) peritoneal fluid was obtained. In group I all abdominal organs were palpated, and sham laparotomy was done. In group II, the distal portion of the cecum was ligated with 4/0 silk, and a full-thickness perforation 0.5 cm in diameter in the cecum was created. In group III, after cecal ligation and perforation as in group II, the gastroepiploic vessels were ligated with 4/0 silk, and total omentectomy was performed. In group IV, only total omentectomy was performed as in group III. In all groups, the abdominal wall was closed with 3/0 nonabsorbable sutures. 2- and 4-hour peritoneal fluid samples were taken using a Pasteur pipette under anesthesia. The following parameters were assessed in the fluid samples: (1) total peritoneal cell count; (2) bactericidal activity of peritoneal fluid, and (3) types of phagocytic cells in the peritoneal fluid.

Microbiology

After 18 h of culture in tryptic soy broths, *Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter freundii, Pseudomonas aeruginosa, Proteus mirabilis,* and *Candida albicans* were isolated from the feces. The microorganisms were inoculated after dilution to 10^4/ml using several culture techniques.

Peritoneal Fluid

Peritoneal lavage was performed by giving 1 ml sterile saline into the peritoneal cavity using a Pasteur pipette. The fluid was collected into centrifuge tubes. The cells obtained from peritoneal lavage fluid were placed on a 1-cm^3 area of Thoma and guiding slides. The cells were stained with Giemsa and counted. Total peritoneal cells and phagocytes were counted (cells/ml). The phagocytic cells were classified and their percentage calculated on Giemsa-stained slides. Fluid samples taken at 0, 2, and 4 h were centrifuged, then passed through a Seitz filter (pore diameter 200 nm), and sterilized. 0.1 ml of fluid containing 10^4 microorganism per milliliter and 0.9 ml of filtered peritoneal fluid were mixed in a tube. The passages from this solution were inoculated into Mueller-Hinton agar at 0, 2, 4, and 24 h. The inoculates were spread over the surface of agar plates in a standard pattern, so that the quantity of bacterial growth could be determined either semiquantitatively or relatively. After each culture was incubated at 37°C for 48 h, the number of proliferating colonies was counted. Bacteriostatic and bactericidal effects of the peritoneal fluid on microorganisms were assessed. This procedure was repeated for each microorganism and the fluid taken from the peritoneal cavity at 0, 2, and 4 h. Control cultures were done for each group.

Statistics

To compare the intragroup and intergroup results, Student's t test and Anova were used, respectively, and $p < 0.05$ was considered to be statistically significant.

Results

Total Peritoneal Cell Counts

Total peritoneal cell counts in all groups at 0, 2, and 4 h are shown in figure 1. According to the baseline count of the sham laparotomy group, the total peritoneal cell count increased after 2 h (p = 0.018) and 4 h (p = 0.006). A significant increase was observed after 4 h as compared with 2 h (p = 0.006).

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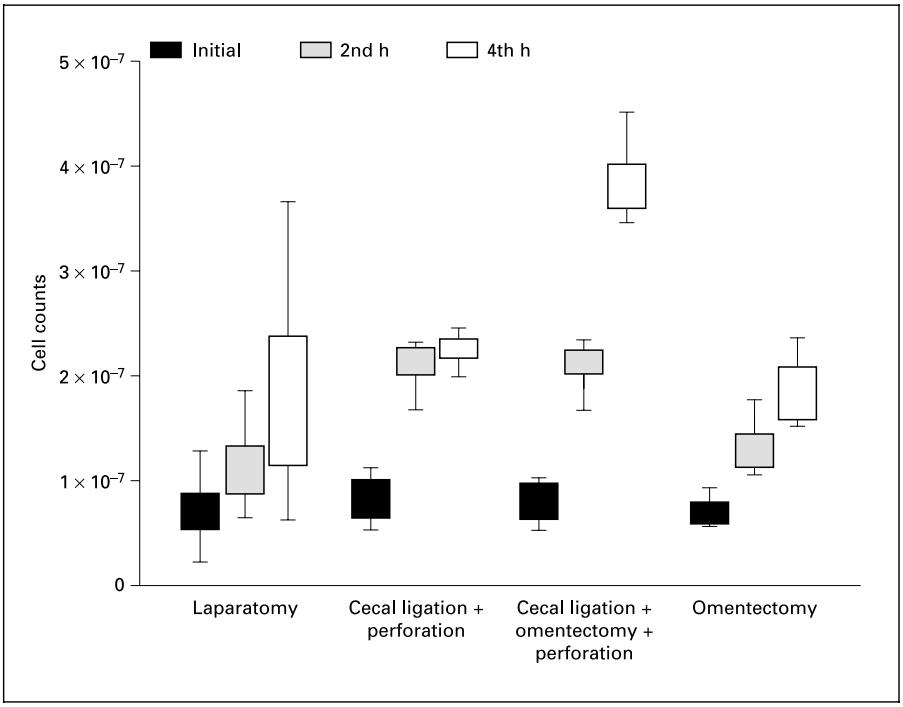


Fig. 1. Mean (±SD) counts of total peritoneal cells as revealed by the Thoma slide method.

A significant increase in total peritoneal cell count in the cecal ligation + perforation group was found after 2 and 4 h when compared with the baseline values (p = 0.0001). There was no significant difference between the values after 2 and 4 h (p = 0.678).

In the cecal ligation + perforation + omentectomy group, the total peritoneal cell counts significantly increased after 2 and 4 h as compared with the baseline values (p = 0.0001). Also the counts after 4 h were considerably higher than after the 2nd h (p = 0.0001).

In the omentectomy group, the total peritoneal cell counts significantly increased after 2 and 4 h as compared with the baseline values (p = 0.0001). Also the counts after 4 h were considerably higher than after 2 h (p = 0.0001).

There was no significant difference between the baseline cell numbers of all groups (p = 0.7). A significant increase was found in all treatment groups as compared with the sham laparotomy group after 2 h (p = 0.0001). After 4 h, there was a significant difference between the groups, but especially striking in the cecum ligation + perforation + omentectomy group (p = 0. 0001).

Bactericidal Activity of Peritoneal Cells

In all groups, the peritoneal fluid was not effective against *P. mirabilis, E. aerogenes,* and *C. freundii.* Also it was observed that proliferation of *K. pneumonia* stopped for 8 h. But it was determined that proliferation in cultures increased after 24 h. A decrease in proliferation was observed in *C. albicans* at the end of 4 and 8 h, and an increase in proliferation was seen at the end of the 24th h. It was also observed that the proliferating colony counts of *E. coli* and *P. aeruginosa* decreased after the 2nd h, and there was no proliferation in the subsequent cultures (table 1).

Table 1. Bactericidal effects of the peritoneal fluid samples obtained from all rats at different time points

Microorganisms	Time, h				
	0	2	4	8	24
E. coli	130	45	–	–	–
P. aeroginosa	420	124	–	–	–
K. pneumoniae	130	100	90	95	excessive
C. albicans	152	184	50	30	140

Classification of the Phagocytes

The cells were classified as macrophages, polymorphonuclear neutrophils, lymphocytes, and mast cells and then counted. Counts and types of cells observed in all groups

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Table 2. Mean counts of peritoneal defense cells by the Giemsa slide method

Group	Time h	Macrophages		Polymorphonuclear neutrophils		Lymphocytes		Mast cells		Total counts of cells (mean ± SD)
		n	%	n	%	n	%	n	%	
Sham laparotomy	0	2.2×10^6	35.7	1.7×10^6	28.3	2×10^6	33	2×10^5	3	$6.1\times10^6\pm1.3\times10^4$
	2	4.3×10^6	43	2.5×10^6	24.5	3.2×10^6	31.1	1×10^5	1.4	$10.1\times10^6\pm6.2\times10^3$
	4	8.1×10^6	40.3	6×10^6	30	5.7×10^6	27.9	4×10^5	1.8	$20.2\times10^6\pm1\times10^4$
Cecal ligation + perforation	0	3×10^6	42.2	2×10^6	28.9	1.9×10^6	28	1×10^5	0.9	$7\times10^6\pm5.8\times10^3$
	2	8.1×10^6	40.5	5.3×10^6	26.4	6.3×10^6	31.3	4×10^5	1.8	$20.1\times10^6\pm6\times10^3$
	4	10.5×10^6	44.6	5.6×10^6	23.6	7×10^6	29.4	6×10^5	2.4	$23.7\times10^6\pm1.3\times10^4$
Cecal ligation + perforation + omentectomy	0	3.3×10^6	45	1.8×10^6	24.6	2.1×10^6	28.5	1×10^5	1.9	$7.3\times10^6\pm1.9\times10^4$
	2	9.8×10^6	47.2	5.2×10^6	25.3	5.4×10^6	25.7	4×10^5	1.8	$20.8\times10^6\pm1.2\times10^4$
	4	20.6×10^6	48.5	11×10^6	26.1	9.8×10^6	23.2	1×10^6	2.2	$42.4\times10^6\pm1.7\times10^5$
Omentectomy	0	1.3×10^6	20.6	1.9×10^6	30.9	2.9×10^6	45.3	2×10^5	3.2	$6.3\times10^6\pm1.2\times10^4$
	2	2.5×10^6	20.6	6.5×10^6	53.6	2.7×10^6	22.6	4×10^5	3.2	$12.1\times10^6\pm1\times10^4$
	4	1.7×10^6	8.9	11.2×10^6	60.9	4.3×10^6	23.3	1.2×10^6	7	$18.4\times10^6\pm1.2\times10^4$

are shown in table 2. In comparison of the groups themselves, it was observed that the macrophage counts significantly increased after 2 and 4 h as compared with baseline values ($p = 0.0001$). An increase was observed in the macrophage counts at 0, 2, and 4 h in intergroup comparisons, and it was especially significant in the cecal ligation + perforation + omentectomy group ($p = 0.0001$). In the omentectomy group, a significant decrease was observed in the macrophage counts between the 2nd and 4th h ($p = 0.0001$).

Discussion

Bacteria are removed from the peritoneal cavity by absorption via diaphragmatic lymphatics [6]. Although the diaphragmatic lymphatics have the principal role by absorption, the omentum is also suggested to have an important role. The fibrinolytic activity of the peritoneal mesothelium is disturbed in conditions such as trauma, ischemia, and infections [5]. As a result of an increase in the vascular permeability, fibrinogen and thromboplastin levels released from the destructed cells increase, and on the other hand the decrease in the fibrinolysis increases the fibrin formation in the abdominal cavity. An increase in the fibrin formation leads to adhesions in omentum, peritoneum, and mobile organs and prevents the extension of infections [1].

The mechanical barrier effect of fibrin prevents the entrance of bacteria into the circulation and leads to abscess formation by destroying the antibiotic and phagocytic activities [7, 8].

It was reported that in rats in which omentectomy was not performed, the microorganisms remained phagocytosed on the omental surface [1]. There are different ideas on the resources of macrophages. A widely accepted concept is that these cells belong to the mononuclear phagocytic system and after passing into the circulation from bone marrow, they remain 1–4 days in the systemic circulation and then enter the peritoneal spaces [1]. Another concept is that these cells do not originate from the bone marrow but from omental milky spots [9]. The production of macrophages by the omentum was shown in vivo [10]. It was found that the macrophages are the most abundant group in the omental milky spots [11]. Agalar et al. [12] applied omentectomy without intra-abdominal infection; they realized a decrease in the macrophage and total peritoneal cell counts. They rationalized this decrease as a result of the removal of milky spots and macrophage-stimulating factor found in omentum. It was shown in a study of Ratajczak et al. [13] that one of the functions of the omentum is stimulation of macrophage colonies. The factor secreted by the omental stromal cells is called colony-stimulating factor 1 which is necessary for production and development of peritoneal macrophages [13, 14]. In this study, there was a significant difference in total peritoneal cell counts at the 2nd h between the cecal

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ligation + perforation and the cecal ligation + perforation + omentectomy groups. But at the 4th h this difference was approximately twofold with respect to the omentectomy group. This is due to a proportionate increase in the macrophage count. In this study, while there was no significant difference between the baseline average total peritoneal cell counts, there was significant difference between the groups at the 2nd h. It is more important that the 4th-hour mean peritoneal cell counts of the omentectomy + cecal ligation + perforation group was twice the average of peritoneal cell counts of the control group and the cecal ligation + perforation group with intra-abdominal infection. We think that a decrease in the fibrinolytic activity [15, 16] resulting from omentectomy causes an increase in the mechanical barrier effect of fibrin, thus an increase in bacteria in the abdominal cavity causes an increase in the number of peritoneal defense cells originating from systemic macrophages with a rebound effect. The macrophage count was higher in the cecal perforation group with intra-abdominal infections as compared with the control group. This can be explained by the presence of omentum and the migration of peripheral macrophages into the abdomen. The increase in macrophage counts was not seen in the omentectomy group when compared with the cecal ligation + perforation + omentectomy group. This can be explained by the stimulation of peritoneal defense mechanisms by intra-abdominal infections.

E. coli and *Bacteroides fragilis* are two of the most commonly isolated pathogens from the cultures obtained during operations, leading to intra-abdominal infections [17, 18]. In the study performed by Bercovici et al. [6], it was shown that peritoneal fluid has bactericidal and bacteriostatic effects against gram-positive and gram-negative strains and fungi. In our study, it was shown that peritoneal fluid has antimicrobial effects during definite time points. In all four groups, it was observed that the proliferation of *E. coli* and *P. aeroginosa* ceased after 4 h. It was also observed that the omentum had no role in peritoneal bactericidal activity against these bacterial species. In all four groups it was observed that the colony counts of *K. pneumonia* and *C. albicans* decreased after the 4th h, but the colonization of both organisms increased after 24 h. We found that the bactericidal activity of the peritoneal fluid against *E.coli* did not change after omentectomy, as Agalar et al. [12] reported.

The killing ability of macrophages and diaphragmatic lymphatic absorption are the first-line peritoneal defense mechanisms [19–22]. In the previous experimental studies, it was observed that macrophages are active initially and that their number stabilized after bacterial occupation [19]. Although the phagocytic potentials of macrophages and polymorphonuclear neutrophils are similar, the macrophages have a dominant role during the early stages of phagocytosis and are present in higher numbers [19, 20]. In this study, in all three groups, until the 4th h, macrophages were found higher in number and ratio as compared with polymorphonuclear neutrophils. But in the omentectomy group, polymorphonuclear neutrophils were found higher in number and ratio with respect to macrophages. We think that the reason for this is the removal of macrophage colony-stimulating factor with omentectomy. While the proportion of the macrophages in the cecal perforation group was 40.5% at the 2nd h, it was calculated as 47.2 and 20.6% in the cecal perforation + omentectomy group and in the omentectomy group at the 2nd h, respectively. In the group in which omentectomy + cecal perforation was performed, although the macrophage resource was subtracted, domination of macrophages continued by the peripheral macrophage migration because of the stimulation by intra-abdominal infection during the earlier stage of phagocytosis. But in the omentectomy group, the macrophage count did not increase because of the lack of intra-abdominal infections.

We conclude that the removal of omentum in the presence of intra-abdominal infections causes the peripherally derived macrophages to take over the defensive role as a compansatory mechanism, thus the peritoneal bactericidal activity against *E. coli*, the major pathogen in intra-abdominal infections, does not change after omentectomy.

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