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KARINIÇI İNFEKSİYONUN DİYAFRAGMA LENFATİKLERİ YOLUYLA SİSTEMİK YAYILIMI: RATLARDA SERUM ENDOTOKSİN VE SİTOKIN SEVİYELERİYLE DEĞERLENDİRME.

SYSTEMIC SPREAD OF INTRAABDOMINAL INFECTION VIA TRANSDIAPHRAGMATIC LYMPHATICS: EVALUATION WITH SERUM ENDOTOXIN AND CYTOKINE LEVEL IN RATS.

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ÖZET: Karınıçi infeksiyonun sistemik yayılımı, cerrahi pratiğinde sık görülen ve hayatı tehdit eden bir durumdur. Bu çalışmada, deneysel peritonit esnasındaki septik cevap oluşumunda lenfatik sistemin rolünü araştırdık. Çalışma eşit olarak üç gruba ayrılan 30 rat üzerinde gerçekleştirildi. Grup 1, "sham" laparotomi. Grup 2, çekum delinmesiyle peritonit oluşturuldu. Grup 3, lenfatik ağızlarını fibrotik reaksiyonla tıkamak için bir tabaka sentetik materyal diyafragmanın abdominal yüzeyine yerleştirildi. Altı hafta sonra önceki gruptaki gibi peritonit oluşturuldu. Çekum delinmesinden altı saat sonra, histopatolojik inceleme, periton sıvısı ve kanın mikrobiyolojik analizi, ve plasma endotoksin ve serum tumör nekroz faktörü (TNF) ölçümleri için örnekler toplandı. Bakteriyel peritonit grup 2 ve 3'teki tüm 20 sıçanda mevcuttu. Kan kültür sonuçları 2. gruptaki 7, 3. gruptaki 2 sıçanda pozitif idi ($p=0.035$). Sırsıyla grup 2 ve 3'teki plasma endotoksin seviyeleri 8.3 ve 1.8 EU/ml ($p<0.001$), serum TNF seviyeleri 432 ve 129 pg/ml ($p<0.001$) ölçüldü. Diyafragma abdominal yüzeyindeki lenfatik ağızlarının tıkanması, deneysel peritonit esnasındaki septik cevap şiddetini anlamlı olarak düşürdü. Sonuç olarak, bakteriler ve endotoksin periton boşluğundan organzmaya lenfatik kanallarla hızla yayılmakta, ve karınıçi infeksiyonun erken döneminde ciddi septik cevabı uyarmaktadır. Karınıçi inbfeksiyonun sistemik yayılımında lenfatik sistemin major rolü vardır.
Anahtar kelimeler: Peritonit, diyafragma lenfatikleri, bakteriyemi, endotoksemi, TNF.

SUMMARY: Systemic spread intraabdominal infection is a common life-threatening condition in surgical practice. In the present study we investigated the role of lymphatic system in the occurrence of septic response during experimental peritonitis. The study was carried out on 30 rats equally separated into three groups. Group 1, sham laparotomy. Group 2, peritonitis was induced with cecal puncture. Group 3, a sheet of synthetic material was placed on the abdominal surface of the diaphragm in order to occlude lymphatic openings by a fibrous reaction. After six weeks peritonitis was created as in previous group. Samples were collected six hours after caecal puncture for: histopatological examination, microbiological analysis of peritoneal fluid and blood, and measurements of plasma endotoxin and serum tumor necrosis factor (TNF) levels. Bacterial peritonitis was present in all 20 animals of groups 2 and 3. Results of blood cultures were positive in 7/10 rats of group 2 and in 2/10 rats of group 3 ($p=0.035$). Mean plasma endotoxin levels were 8.3 and 1.8 EU/ml, and mean serum TNF levels 432 and 129 pg/ml in groups 2 and 3 respectively ($p<0.001$). The occlusion of lymphatic openings on the abdominal surface of the diaphragm has significantly reduced the intensity of septic response during experimental peritonitis. We concluded that bacteria and endotoxin have promptly disseminated from the peritoneal cavity through lymphatic channels, and induced severe septic response in the early period of intraabdominal infection. The lymphatic system has a major role in systemic spread of intraabdominal infection.
Key words: Peritonitis, diaphragmatic lymphatics, bacteremia, endotoxemia, TNF.

The systemic spread of peritoneal infection is always a life-threatening condition urging to emergency interventions. Dissemination of bacteria and bacterial cell products to the bloodstream worsens the fate of such patients. Bacteraemia and endotoxemia lead to significant cytokine release called the septic response. Recent studies have demonstrated that lymphatic system is the most important route from the peritoneal cavity to the blood. Eighty percent of peritoneal fluid is cleaned by diaphragmatic lymphatics [1-3]. It has been shown experimentally that blockage of transdiaphragmatic lymphatic absorption reduces the mortality from peritonitis [2,4]. The presence

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of bacterial cell products in the blood, such as endotoxin, creates more severe systemic response than do bacteria itself, which is designated by over expression of cytokines. In this study, we aimed to investigate transdiaphragmatic transport of infected peritoneal fluid by lymphatic channels, and the development of bacteraemia, endotoxemia, and septic response.

MATERIAL - METHOD

The study was carried out on 30 Wistar-albino rats which were equally separated into three groups.

Group 1 : General anaesthesia was induced with ether. Abdominal cavity was explored through a midline incision, which was then closed with 3/0 silk running suture.

Group 2 : After anaesthetic with ether, the abdomen was opened with a midline incision; the caecum was then perforated with the tip of no 11 scalpel and the abdomen was closed with 3/0 silk running suture.

Group 3 : Six weeks before the experiment the rats were operated on. General anaesthesia was induced with ketamine 100 mg/kg intramuscularly (Ketalar, Parke-Davis, Eczacıbası, Istanbul). The abdomen was explored through a midline incision. A sheet of polyester (Mersilene) mesh was placed on the abdominal surface of the diaphragm to block lymphatic absorption by a fibrous reaction. The mesh was sutured with 5/0 polydioxanone (PDS, Ethicon) on the abdominal surface of the diaphragm. The abdominal incision was closed with 3/0 silk running suture. After six weeks, peritonitis was created by caecal puncture as in the previous group.

Collection of samples: Samples were collected six hours after the induction of peritonitis or sham laparotomy. All animals were anaesthetised with an intramuscular injection of ketamine 100 mg/kg. To obtain blood abdominal aorta was dissected through a midline laparotomy, and a 20 G cannula was inserted.

Specimens for aerobic microbiological examination were taken from the peritoneal cavity with sterile dry cotton-tipped swabs, and 0.5 ml blood was drawn from the abdominal aorta. Specimens were transported to the laboratory.

Plasma endotoxin concentration was measured in 1 ml of blood that was drawn from the abdominal aorta and mixed in a sterile tube with 100 IU of heparine (Liquemine, Roche). Plasma was separated immediately and frozen at -70°C until processed semi-quantitatively using Limulus amoebocyte lysate (LAL) test (E-Toxate, Sigma, USA). Endotoxin level was expressed as EU/ml.

Serum Tumour necrosis factor alpha (TNF) concentration was measured in 1 ml of blood that was drawn from the abdominal aorta and serum was separated immediately and frozen at -70°C until processed with ELISA method using TNF EASIA (enzyme amplified sensitivity immunoassay). Serum TNF level was expressed as pg/ml.

Animals were killed with an overdose of ether. The diaphragm were removed and examined for histopathological changes secondary to the mesh, depending on the formation of fibrous bands and the number of fibrous bridges between the liver and the diaphragm.

Data are presented as mean and standard deviation, and statistical analysis was made with Student's t test and Fisher's exact test, as appropriate. P less than 0.05 was accepted as significant.

RESULT

All animals were alive six hours after cecal puncture. Macroscopic findings were as follows at relaparotomy: In group1; No gross changes. In groups 2 and 3; purulent exudate due to intraabdominal infection. In group 3; firm adhesions between the liver and diaphragm secondary to the prosthetic material. Histopathologic examination showed strong fibrous reaction in all animals of the group 3. Aerobic culture of peritoneal fluid and of blood was sterile in group 1. We determined intraperitoneal bacterial growth in all animals of groups 2 and 3. Microbes mostly encountered were gram-negative aerobes such as E. Coli, Klebsiella and proteus. Results of microbiological analysis of blood showed significantly less positive blood cultures ($p<0.0035$) in group 3 when compared with group 2 (Table 1). Mean plasma endotoxin and serum TNF levels were significantly different beetween two groups with peritonitis, in favor of the group 3 in which transdiaphragmatic lymphatic absorption was occluded by fibrosis ($p<0.001$; Table 2).

Table 1. Results of microbiological analysis: positive aerobic cultures.

Groups	No of rats	Peritoneal fluid	Blood
1-Control	10	0	0
2-Peritonitis	10	10	7*
3-Lymphatic occlusion	10	10	2*

*p=0.035 (Fisher's exact test)

DISCUSSION

Many studies have pointed out absorptive functions of diaphragmatic lymphatic channels. Peritoneal fluid is cleared mainly through multiple lymphatic stomata on the

Table 1. Results of plasma endotoxin and serum TNF measurements.

Groups	No of rats	Endotoxin (EU/ml)	TNF (pg./ml)
1-Control	10	0.25±0.11	66± 18
2-Peritonitis	10	8.3± 1.8	432± 102
3-Lymphatic occlusion	10	1.8± 0.57	129± 43
		*P<0.001	*p<0.001

*p between groups 2 and 3.

abdominal surface of the diaphragm [1,2,3,5,6]. In a previous study we demonstrated experimentally the role of lymphatic system on systemic effects of peritonitis, with changes of body oxygen kinetics [7]. In the present study we have investigated the role of lymphatic system on the transport of bacteria and bacterial products from the peritoneal cavity to the blood.

Our microbiological analysis showed that gram-negative bacterial peritonitis was achieved in all animals with cecal puncture. Peritonitis secondary to cecal perforation is rapidly fatal in rats, and half of the animals die within 12 to 18 hours after the outset of infection [8,9]. We collected samples at 6th hour after the induction of peritonitis, before fatal outcome. In order to determine the role of lymphatic system on the dissemination of peritoneal infection, absorption of peritoneal fluid has been blocked with various methods. We aimed to block this lymphatic absorption by inserting braided synthetic mesh that would cause a fibrous reaction between the liver and diaphragm. Our histopathological findings confirmed that this reaction was achieved in the group 3.

Results of blood cultures in groups 2 and 3 demonstrated that the occlusion of transdiaphragmatic absorption significantly reduced bacteraemia secondary to intraabdominal infection. Dumont et al [4]have also reported that the blockade of the transport of microbes by lymphatics reduces significantly the results of positive blood cultures. Detection of living bacteria in lymph of the thoracic duct 6, and in blood 12 minutes after inoculation of bacteria into the peritoneal cavity, confirms the importance of the transport of peritoneal fluid by lymphatic system [2,3]. Microorganisms in the peritoneal cavity and in portal venous circulation are under control of host phagocytic cells and of the reticuloendothelial system. Microbes can bypass this phagocytic activity by entering the circulation directly through the lymphatic channels, and thereby create bacteraemia.

Bacterial cell products (e.g., endotoxin) have been accepted as main factors responsible from septic effects during gram-negative bacterial infections. Endotoxin can gain access to the systemic circulation and activate a generalised host response to the infectious events. Our findings of severe endotoxemia six hours after perforation of the cecum showed that bacterial cell products promptly disseminated from the peritoneal cavity to systemic circulation even in early period of intraabdominal infection. Lundblad [9] and Eskandari [10] have also reported that plasma endotoxin amount reaches to its peak level four to eight hours after caecal puncture. In the present study the occlusion of lymphatic openings on the abdominal surface of the diaphragm has reduced significantly plasma endotoxin level. Significant difference of plasma endotoxin levels between groups 2 and 3 revealed that endotoxin was transported intensively by lymphatic system from the peritoneal cavity to the blood. Recent reports have pointed out that in the early period of bacterial peritonitis, very high endotoxin levels has been detected in the lymph of the thoracic duct. A fistula of the thoracic duct draining lymph flow, has significantly reduced the intensity of endotoxemia [11,12]. The amount of endotoxin that is transported by the portal vein never exceeds the filtrating capacity of the normal liver. High endotoxin cleaning capacity of the rat liver, reported to be 1.5μg/ g liver per hour [13]. Microbial cell products bypassing the hepatic filtration system by reaching the blood circulation via lymphatic system can elevates plasma endotoxin level.

Systemic response created by bacteria and its products is called septic response which refers to a condition of systemic extention of a process beyond the local focus [14,15]. In our study, the induction of septic response due to the dissemination of intraabdominal infection was reflected with serum TNF levels, the most known cytokine which is a mediator of systemic inflammatory response. Serum TNF levels in groups 2 and 3 showed a close parallelism with plasma endotoxin levels. Both plasma endotoxin and serum TNF levels were significantly elevated after peritonitis, and reduced with the occlusion of lymphatic absorption. Our results showed that endotoxemia, in the early period of intraabdominal infection was mainly due to the transport of endotoxin by lymphatic system, and the intensity of septic response was significantly reduced by the blockade of the transport by lymphatic system.

In conclusion, our results of bacteraemia and endotoxemia showed the detrimental effects of transdiaphragmatic absorption of infected material from the peritoneal cavity. The transport of microbes and their products by lymphatic system increased the intensity of septic response. The septic response could be reduced by occlusion of lymphatic openings on the abdominal surface of the diaphragm.

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