

Transdiaphragmatic blockage of the lymphatic may reduce bacterial translocation



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PURPOSE: Bacterial translocation is a spread of living and non-living bacteria and bacterial end-products, passing over the mucosal barrier distending mesenteric lymph nodes, blood and viscera. DuraSeal (polyglycolic acid glue) has adhesive power and it usually used in brain or spinal surgery. We aimed to show the barrier system effect using experiments, in which intraabdominal infection induced and translocation from diaphragm to thorax is researched.

METHODS: We used 90 Wistar Albino male rats weighing 200-250 grams in our experiment. Laparotomy was performed to all groups. After laparotomy, intraperitoneal 1 cc physiological saline was given in Group I (control). 1×10^9 cfu/ml/kg (colony forming unit/mililiter/kilograms) *E. Coli* was injected intraperitoneally in Group II. In Group III, DuraSeal® (Confluent Surgical, Inc., Waltham, MA) was sprayed on the diaphragm. After waiting for approximately 2 minutes to see the barrier effect, 1×10^9 cfu/ml/kg *E. Coli* was injected intraperitoneally.

RESULTS: For the samples taken intraperitoneally, 100% breeding was determined in all groups except Group I. While no positive staining was observed in the thoraxes of the rats in Group I and Group III at the first hour, the positive staining ratio in the Group II was 70%. The positivity ratio of Group II was 80% and the ratio was 50% in Group III at the third and sixth hours. Regarding hemoculture *E. Coli* positivity, there was no proliferation in the hemoculture samples of Group I at all time periods, whereas it was positive in the other groups excluding the first hour.

CONCLUSIONS: The synthetic hydrogel DuraSeal®, which was designed to prevent postoperative fibrosis and air leakage, was able to partially block the translocation of the bacteria to the thorax via lymphatic or directly.

KEY WORDS: Abdomen, Bacterial translocation, Polyglycolic acid glue, Thorax

Introduction

The gastrointestinal system is an important resource of bacteria. The intestinal immunological system and epithelial cover functioning are effective barriers against intraluminal endogen bacterial invasion and systemic

spreading, in which intraperitoneal or hematological spreading of the existent bacteria may threaten vitality^{1,2}. Bacterial translocation is described as the spread of living and non-living bacteria and bacterial end-products, passing over the mucosal barrier distending mesenteric lymph nodes, blood and viscera^{1,3,4,5}. Although the peritoneal surface has a diffusion capacity for water and water-soluble low molecular weight particles, absorption process is only via diaphragmatic lymphatic system. Reabsorbed particles are taken up to the systemic circulation^{1,5}. There are some peritoneal defensive mechanisms interrupting this situation.

DuraSeal (Confluent Surgical, Waltham, MA) is a polyglycolic acid glue and demonstrates adhesive power^{6,7}. It use for the repair of dural defects either in brain or spinal surgery. It reduces the incidence of postoperative incisional CSF leak after posterior fossa

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surgery and in April 2005, the first United States Food and Drug Administration approved dural sealant, DuraSeal (Confluent Surgical, Waltham, MA), became available for cranial use ^{6,8}.

The aim of this study was to investigate the barrier system effect in an experimental animal model, in which intraabdominal infection was induced and translocation from diaphragm to thorax was evaluated.

Materials and Methods

90 Wistar Albino male rats weighing 200-250 grams were recruited for the experiment that was carried out at the Experimental Research Laboratory of Istanbul University, Cerrahpasa Medical School. The study was approved by the ethical committee of Cerrahpasa Medical School. 30 rats were randomly assigned to one of three groups including:

GROUP I: Laparotomy was performed and intraperitoneal 1 cc physiological saline was given. 3.0 silk sutures were used while closing the abdominal wall. (Control) (n=30).

GROUP II: Laparotomy was performed and 1×10^9 cfu/ml/kg (colony forming unit/mililiter/kilograms) E. Coli was injected intraperitoneally. 3.0 silk sutures were used while closing the abdominal wall (n=30).

Group III: Laparotomy was performed and then DuraSeal® (Confluent Surgical, Inc., Waltham, MA) was sprayed on the diaphragm. After waiting for approximately 2 minutes to see the barrier effect, 1×10^9 cfu/ml/kg E. Coli was injected intraperitoneally. The abdominal wall was sutured by 3.0 silk (n=30).

Surgical procedure:

The rats were anesthetized by intramuscular 100 milligram/kilogram Ketamine HCL (Ketalar flacon, Eczacıbaşı) injection. Abdominal wall was cleaned by Betadine solution for antiseptis. Sterilized instruments were used individually for each rat. Following cutaneous and subcutaneous incisions of 3-cm in length, laparotomy was made.

Microbiological analysis:

Bacterial inoculum was extracted in the microbiology laboratory of Istanbul University, Cerrahpasa Medical School. Four E. Coli extractions, two of which were derived from feces and the other two from urine, in the microbiology laboratory, were injected into the peritoneums of four different Wistar albino rats as 1×10^9 cfu/ml/kg in units. The strain, which was able to create peritonitis and killed the rat in 6-8 hours, was identified. Then this strain was cloned in Tryptic Strain Broth (Bacto®) and used for the experiment. The strain to use in inoculation was resolved to form a suspension by using physiological saline. The concentration index was calculated using a spectrophotometer. In addition, E.

Coli strain was prepared by utilizing Mc. Farland standard tubes, in units of 1×10^9 cfu/ml. The suspension (1×10^9 cfu /ml E. Coli) was injected intraperitoneally to the Groups II and III as 1 ml/200g/rat.

All the rats in each group were divided into three subgroups (n=10) and sacrificed at the 1st, 3rd, and 6th hours accordingly. Peritoneal and then pleural samples were taken, spread over the slides. Immediately afterwards, the thorax was opened and the smear was collected; 1 ml of blood was taken by intracardiac puncture and cultivated in an anaerobe culture for hemoculture results. Then the samples were incubated for 24 hours at 37 °C in an incubator.

The smears taken from both the peritoneum and the pleura by the swabs were spread on the slides and dried by passing through flame. Slides were examined after Gram staining. The microorganisms observed were identified as Gram-positives or Gram-negatives.

The blood samples taken from the rats by intracardiac puncture were inoculated into cultures. Afterwards, passages were taken from the hemoculture tubes for endo-and chocolate-like agars, and left for incubation for 24 hours at 37 °C. Plates were then evaluated to see if there were any breeding of E. Coli, which has a green reflection. For identification, the E. Coli triple diagnostic test was used to see whether the colonies were E. Coli or not; and observed as affirmative.

Statistical Analysis:

The results were evaluated with the SPSS (Statistical Package for Social Sciences) for Windows 10.0 software. For data comparison, the Kruskal Wallis test was chosen, whereas the Wilcoxon sign test was preferred for the intra-group comparisons of the parameters. The Results were considered to be at 95% of the confidence interval with a p value of <0.05.

Results

The first hour thoracic positivity revealed a high level of difference in terms of statistical significance ($p < 0.01$). While no positive staining was observed in the thoraxes of the rats in Groups I and III at the first hour, the positive staining ratio in the Group II was 70% (Table I).

Regarding the third hour thoracic positivity, a significantly high level of difference was found ($p < 0.01$). Whilst no positive staining was observed in the thoraxes of the rats in Group I at the third hour, the positivity ratio of Group II was 80% and the ratio was 50% in Group III (Table I).

The sixth hour thoracic positivity indicated that there was a significantly high level of difference ($p < 0.01$). Whilst no positive staining was observed in the thoraxes of the rats in Group I at the sixth hour, the positivity ratio of Group II was 80% and it was 50% in Group III (Table I).

TABLE I - *E. Coli* positivity in thorax

Thorax		Group I		Group II		Group III		Stat. of Test; p
		N.	%	N.	%	N.	%	
1st hour	Negative	10	100	3	30,0	10	100	KW:17,652; p:0,001**
	Positive			7	70,0			
3rd hour	Negative	10	100	2	20,0	5	50,0	KW:12,860; p:0,002**
	Positive			8	80,0	5	50,0	
6th hour	Negative	10	100	2	20,0	5	50,0	KW:12,860; p:0,002**
	Positive			8	80,0	5	50,0	

TABLE II - *E. Coli* positivity in abdomen

Abdomen		Group I		Group II		Group III		Stat. of Test; p
		N.	%	N.	%	N.	%	
1st hour	Negative	10	100					KW:29,000; p:0,001**
	Positive			10	100	10	100	
3rd hour	Negative	10	100					KW:29,000; p:0,001**
	Positive			10	100	10	100	
6th hour	Negative	10	100					KW:29,000; p:0,001**
	Positive			10	100	10	100	

TABLE III - *Hemoculture* positivity

Hemoculture		Group I		Group II		Group III		Stat. of Test; p
		N.	%	N.	%	N.	%	
1st hour	Negative	10	100	10	100	10	100	KW:0,000; p:1,000
	Positive							
3rd hour	Negative	10	100					KW:29,000; p:0,001**
	Positive			10	100	10	100	
6th hour	Negative	10	100					KW:29,000; p:0,001**
	Positive			10	100	10	100	

No statistically significant differences were observed in Group II, according to the positivity ratio in the first hour compared to the results of the third and sixth hours ($p>0.05$). (Table I). A significantly high level of difference was found between groups II and III at all time periods ($p<0.01$).

For the samples taken intraperitoneally, 100% breeding was determined in all groups except Group I at all time periods (Table II). Regarding hemoculture *E. Coli* positivity, there was no proliferation in the hemoculture samples of Group I at all time periods, whereas it was positive in the other groups excluding the first hour (Table III).

Discussion

Bacterial translocation is the migration of bacteria or bacterial products from the intestinal lumen to the mesenteric lymph nodes or other extraintestinal organs and sites^{5,9}. Especially when a breakdown in the integrity of the intestine and the change of the intestinal flora in favor of pathogen microorganisms occurs, bacteria and the bacterial materials pass through the mucosal barrier and disseminate into the mesenteric lymph nodes, liver, spleen and/or bloodstream^{1,2,5,10}. Bacteria, endotoxins and bacterial end-products show their systemic affect by diffusing through the lymphatic system¹⁰. In a normal,

healthy individual, gut originated bacteremia and sepsis do not occur because the host has multiple defense mechanisms to prevent the bacteria and their products from crossing the mucosal barrier and spreading to systemic tissues^{9,10}. Bacterial translocation is associated with pathologic conditions such as trauma, peritoneal inflammation, changes in microflora, intestinal obstruction, parenteral nutrition, intestinal epithelium damage, ionizing radiation and antibiotic therapy^{1,3,4}. When bacterial invasion is made, the peritoneum deals with an infection in three ways²: first, the direct absorption of bacteria into the lymphatics via the stomas of the diaphragmatic peritoneum; second, the local destruction of bacteria through phagocytosis by either resident macrophages or polymorphonuclear granulocytes attracted to the peritoneal cavity; and third, the localization of the infection in the form of an abscess¹¹. According to Balsan et al.¹⁰; there are two major routes that bacterial compounds might gain access to the systemic circulation: through the enteric venous system to the portal vein or following lymphatic drainage. The lymphatic route was investigated and convincing evidence suggested that it might be the principal pathway of translocation¹². Experimental and clinical studies detected viable bacteria in lymph nodes^{2,13}. As well, all those particles are absorbed by the terminal lymphatic, which are called lacunas and found at the peritoneal surface of the diaphragm; and stomas, which are in between mesothelial cells and adjacent to each other, maintain the passage of the materials into the lymphatic^{2,11,14}. An interesting association is that of lung injury observed in the adult respiratory distress syndrome (ARDS) occurring in septic patients and its correlation to translocation^{10,12}. This situation may be explained with this theory; since the mesenteric lymph flows through the thoracic duct, reaches the systemic circulation through the subclavian vein draining to superior vena cava, then to the left atrium and finally to the pulmonary artery and pulmonary vasculature. Thus, the lungs are the first organ to receive the lymph drainage from the gut^{13,15}. In another theory, after passing through subserosal lymphatic plexuses, particles interfuse to the systemic circulation by the way of diaphragmatic lymphatic capillaries and then the anterior mediastinum and thoracic duct^{13,14}. Therefore, the reduced translocation of the bacteria to the thorax in the present study was assumed to be in accordance with this latter theory. It is emphasized that the cicatrization of the diaphragm in rats changes the kinetics of the monoclonal antibodies given intraperitoneally. Hence, it assists the idea advocating that transdiaphragmatic lymphatic absorption is an important route for transportation through the peritoneal cavity¹⁵. Studies indicate that the diffusion of the particles to the lymphatic is excessive because of the widening in the openings on the diaphragm due to the increase in the intraabdominal pressure^{12,14}. Gürleyik E. et al.¹⁶ showed

that animals, in which transdiaphragmatic drainage was obstructed, had fewer positive blood cultures and better body oxygen balance during peritonitis, indicating that the blockage of transdiaphragmatic lymphatic absorption of the peritoneal contents reduced systemic inflammatory response syndrome.

The physical properties of DuraSeal (Confluent Surgical, Waltham, MA), a hydrogel dural sealant, make it an effective adjunct to dural closure. The sealant is composed of two solutions, a polyethylene glycol (PEG) ester solution and a trilycine amine solution. When mixed together, the precursors cross link to form the hydrogel sealant. The hydrogel implant is absorbed in approximately 4 to 8 weeks. It demonstrates strong adhesive power without producing neurotoxicity⁶⁻⁸. It is intended for use as an adjunct to sutured dural repair during cranial surgery to provide watertight closure. Herein, we evaluate the possibility of DuraSeal as a substitute in the blockage of transdiaphragmatic lymphatic absorption. Moreover, despite the peritonitis, in the group which had the lymphatic blockage by infradiaphragmatic cicatrization procedure, significant differences were found regarding bacteremia and mortality results. These results indicate that diaphragmatic lymphatic absorption may have a crucial role in the early periods of the systemic propagation of the intraperitoneal infection and pathogenesis of sepsis. In conclusion, the synthetic hydrogel Duraseal®, designed to prevent postoperative fibrosis and air leakage, was able to partially block the translocation of the bacteria to the thorax via lymphatic or directly. These favorable results indicate that this material may be beneficial for use in patients at high risk groups. Despite the gradually increasing experimental evidences showing its important role in the pathogenesis of sepsis, the clinical importance of the diaphragmatic lymphatic way is not clear yet. There is obviously a need for a considerable amount of further research to elucidate this point.

Riassunto

La traslocazione batterica è la diffusione di batteri vivi o morti e dei loro prodotti di degradazione, che superano la barriera mucosa e diffondono ai linfonodi mesenterici, nel sangue e nei visceri.

Il DuraSeal (colla di acido poliglicolico) è dotato di potere adesivo ed è spesso usato in neurochirurgia, dell'encefalo e del midollo. Con questo studio ci siamo proposti di dimostrare sperimentalmente l'effetto del sistema barriera, con una ricerca della traslocazione dal diaframma al torace di una infezione intra-addominale provocata.

Per questi esperimenti abbiamo usato 90 ratti Wistar Albini, di sesso maschile, del peso di 200-250 g.

Il Gruppo I è stato lasciato come gruppo di controllo.

Nel Gruppo III abbiamo eseguito una laparotomia ed spruzzato sul diaframma il DuraSeal® (Confluent Surgical, Inc., Waltham, MA), aspettando circa 2 minuti per verificare la costituzione della barriera; quindi è stato iniettato nel cavo peritoneale 1x10⁹ cfu/kg di *Escherichia Coli*. Dopo la laparotomia nel Gruppo II è stato soltanto iniettato nel cavo peritoneale 1x10⁹ cfu/ml/kg di *Escherichia Coli* preparato preventivamente. Per i campioni prelevati dal peritoneo, in tutti i gruppi eccetto il Gruppo I è stata verificata il 100% di proliferazione batterica. Mentre non è stata osservata nel torace nessuna colorazione positiva per la presenza di batteri nei ratti dei Gruppi I e III alla prima ora, la percentuale di colorazione positiva nel II Gruppo è stata del 70%. Alla terza ora questa positività di colorazione nel Gruppo II era del 80% e nel Gruppo III del 50%. La positività toracica alla sesta ora indicava un ulteriore aumento di dislivello percentuale di significato statistico. In conclusione l'idrogel sintetico Duraseal®, utilizzato per prevenire la fibrosi postoperatoria e le perdite aeree postoperatorie, era in grado di bloccare parzialmente la traslocazione batterica al torace per via linfatica o direttamente.

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