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ORIGINAL ARTICLE

Is there any change in the pattern of bacterial translocation with increased time of the obstruction?

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Abstract

Introduction: Bacterial translocation is defined as the passage of bacteria from intestinal tract to the extraintestinal organs such as the peritoneum and blood circulation. The aim of this study is to examine bacterial translocation (regarding type of bacteria and effect of time of obstruction on bacterial translocation) from intestinal lumen to the peritoneum and viscera in acute, simple mechanical, small bowel obstruction in rats.

Methods: In this cohort study, thirty female Wistar rats were divided into three groups with two subgroups, each subgroup containing 5 rats. The 1st group consisted of two sham-operated and non-operated control subgroups. The 2nd group was the IO-24 group, and the 3rd group was the IO-48 group in which the interval between producing intestinal obstruction and the second laparotomy was 24 h and 48 h respectively. Each subgroup was divided into two subgroups of partial and complete obstruction. The data was analyzed using Fisher's exact test and K2 test in SPSS.

Results: The most common types of bacteria were E. coli in aerobic culturing and bacteroid in anaerobic culturing. However, as the time of obstruction increased, the pattern of bacterial translocation changed to anaerobic bacteria.

Conclusions: Our study showed that with increased time of obstruction, pattern of bacterial translocation changed from aerobic to anaerobic. Enterococci were the most common type of bacteria in an aerobic group.

Key Words: Intestinal obstruction; Bacterial translocation; Rats

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Introduction

Recent extensive research has recognized a function of gastrointestinal tract other than simple digestion, absorption and excretion of food, namely intestinal barrier function, which describes the ability of the gut epithelium to separate potentially harmful luminal contents such as bacteria and endotoxins from the closely regulated internal milieu of the human body [1].

Intestinal barrier dysfunction can lead to the invasion of intestinal anti-genes and toxins to blood circulation. It then causes the release of systemic mediators to the circulation, which in turn, activate or stimulate cellular immune system and finally result in systemic inflammation and multiple organ failure [2]. Bacterial translocation is most often found with intestinal manipulation, obstruction, open fractures and burns; it may be also observed in cases with sepsis, multiple trauma, and ileus [3].

The aim of this study is to evaluate bacterial translocation (in terms of type of bacteria and effect of time of obstruction in bacterial translocation) from intestinal lumen to the peritoneum and viscera in acute simple mechanical small bowel obstruction in rats.

Methods

The experiment was conducted on 30 female Wistar rats weighing 200-250 g. The sample size was estimated by the Fisher's exact test for the level of significance set for five percent and the power of at least 80 percent. The NCSS & PASS software was used to estimate the sample size. Therefore, we had 30 rats. The animals were kept in a 25±20°C temperature in Mashhad University's animal lab with a 12 hr light/dark cycle. They were fed with standard diet and tap drinking water *ad libitum* for 48 hours and checked every 24 hour. The rats were randomly assigned into six groups (three main groups with two subgroups in each of them) (n=5 in each subgroup).

Group 1 or the control group consisted of two subgroups; in the 1st subgroup, the rats were only controlled, and nothing else was done; in the 2nd subgroup or sham, laparotomy only (LO) was performed, and the abdomen was closed without performing any further surgical procedure.

Group 2 was the IO-24 group in which the interval between producing intestinal obstruction and the second laparotomy was 24 hours. The 1st subgroup of IO-24 (i.e., CIO-24) underwent complete intestinal obstruction, while in the 2nd subgroup (i.e., PIO-24), partial intestinal obstruction was exercised. Then, the abdomen was closed and again laparotomy was performed within the next 24 hours.

And group 3 was the IO-48 group in which the second laparotomy was performed 48 h after producing intestinal obstruction. This group contained the 1st subgroup of complete intestinal obstruction (CIO-48) and the 2nd subgroup of partial intestinal obstruction (PIO-48).

Sampling of blood and peritoneal fluid was performed to determinate the type of bacteria in all groups after 24 and 48 hours respectively.

Surgery technique

At first, the rats underwent general anesthesia with Ketamine and Xylazine. A mixture of 0.2 mg Ketamine 10% (=0.2 cc) and 1 mg Xylazine 10% (=0.1 cc) was intraperitoneally injected using an insulin syringe. In 2-3 min, the rats underwent generally anesthetized. Heart pulse rate and respiratory rate were monitored during surgery.

The abdomen was gently shaved and then the rats were transferred to the surgery table. With the rats in supine position and under sterile condition, the abdomen was opened through a midline incision. At first, the small intestine was evaluated for any anatomic abnormality which may exclude the rats from the study. Terminal ileum was detected through cecum, and a defect was made in the mesenteryand then a complete or partial obstruction was performed using laparoscopic clip (Horizon Company) which was applied at approximately 2 cm to the ileocecal valve.

In complete obstruction, the clip was fastened as tight as to create complete obstruction with no pressure on intestinal vessels, which may result in ischemia. For partial intestinal obstruction, the small bowel at 2 cm proximal to the distal ileum was incompletely closed using a laparoscopic clip. This type of closure allows some liquid contents and gas to pass through the point of obstruction, whereas complete obstruction impedes the passage of all bowel contents. Then, intestines were returned to the abdomen and the wound or incision was sutured with nylon 3-0 at two layers. The sterile condition was maintained throughout the study.

No rats died during the surgery. The rats were returned to the cage for recovery. Then, the rats were fed by similar water and food.

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Environmental light was adjusted according to the condition (12-h light, 12-h darkness). Then, the rats were again transferred to the laboratory for the second stage of surgery.

Mortality was recorded and the rats were generally anesthetized under the same conditions as the previous surgery. Under sterile conditions with the rats in supine position, the abdomen was opened through the previous incision. Upon entering the abdominal cavity, the intestines were evaluated in terms of the presence of dilatation, which was suggestive of the success of the first surgery.

The intestines were also evaluated in the view of perforation, ischemia signs, and necrosis. In case of any of these signs, the rats were excluded from the study. No rat was excluded from the study since none of the signs were observed. Normal saline was poured into the abdominal cavity using a tiny sampler, and then the samples were taken by the sampler. This sampling was also performed in dead rats. The samples were transferred to blood agar plates for aerobic and anaerobic culturing. Then, a clamshell thoracotomy was performed and blood was directly drawn from the heart and cultured in tryptic soy broth (TSB). The samples were incubated in aerobic and anaerobic conditions for 48 and 96 hours respectively. The cultures were evaluated after incubation. Type of bacteria was detected and the number of colonies was counted on plates. Type of bacteria grown on TSB was also detected.

The data were analyzed through descriptive statistics, including frequency, mean and standard deviation, etc. In addition, the Fisher's exact test was used to compare the groups and subgroups based on different variables.

Results

All the samples of blood cultures were positive except for those of the control group. Because of the liquidness of culture media, it was only possible to determine the type of bacteria but not to enumerate it. The results of blood cultures are shown in Table 1.

Evaluation of the aerobic and anaerobic samples of peritoneum culture showed that all the samples were positive, regardless of their type. The most common aerobic bacteria was E-coli (in IO-24 and IO-48, both partial and complete), while the most common type of anaerobic bacteria was bacterioid. The results are shown in Table 2. It indicates a significant difference between the rate of aerobic and anaerobic bacteria in the peritoneal culture of each subgroup ($p<0.01$).

Table 1: Results of blood culture

Group	Result	Type of bacteria
Control	Negative	Negative
LO	Negative	Negative
PI0-24	Positive	E. coli, bacterioid,
CI0-24	Positive	E. coli, bacterioid
PI0-48	Positive	E. coli, bacterioid
CI0-48	Positive	Enterococci, E. coli

Table 2: Results of peritoneal and blood culture and the mean bacterial growth rate

Group	Peritoneum		P-value	Blood
	Anaerobic	Aerobic		
Control	Negative	Negative	--	Negative
Sham	2	3	--	Negative
PI0-24	203±31	426±22	0.0002	Positive
CI0-24	459±53	700±36	0.0011	Positive
PI0-48	83±19	228±34	0.0011	Positive
CI0-48	46±13	167±26	0.0007	Positive

The type and count of bacteria such as the aerobic and anaerobic were evaluated in all the groups. The Fisher's exact test showed a significant difference in the ratio of aerobic bacteria in 4 subgroups ($p<0.0001$). The results shown in Table 3 indicate a significant difference in all types of bacteria.

Finally, the overall mortality was reported in each group. The highest rate of mortality was found in CIO-48 group (Table 4). Comparison of the survival rate in IO-24 group using Fisher's exact test showed no significant difference between type of intestinal obstruction and mortality status (dead and survived rats) ($p=0.4444$). Also, no significant difference was found between type of intestinal obstruction and mortality status in IO-48 ($p=0.206$).

The culture results of the samples taken from the dead rats were reviewed which are shown in Table 5.

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Table 3: The mean and standard deviation of bacteria (blood and peritoneum)

Type of bacteria	CI0-48		PI0-48		CI0-24		PI0-24		P-value*
	Anaerobic Freq. (Percent)	Aerobic Freq. (Percent)	Anaerobic Freq. (Percent)	Aerobic Freq. (Percent)	Anaerobic Freq. (Percent)	Aerobic Freq. (Percent)	Anaerobic Freq. (Percent)	Aerobic Freq. (Percent)	
E. coli	43 (35.8%)	23 (3.4%)	304 (31%)	279 (83.3%)	1026 (36.6%)	1180 (85.7%)	787 (24.9%)	856 (84.2%)	0.003
Entrococci	39 (32.5%)	550 (80.5%)	74 (7.6%)	43 (12.8%)	142 (5.1%)	141 (10.2%)	104 (3.3%)	129 (12.7%)	<0.0001
Bacterioid	50 (7.3%)	0	541 (55.2%)	0	1636 (58.3%)	0	1235 (39.1%)	0	<0.0001
Pseudomonas	38 (31.7%)	60 (8.8%)	0	0	0	56 (4.1%)	0	27 (2.7%)	<0.0001
Miscellaneous	0	0	61(6.2%)	13(3.9%)	0	0	1035 (32.7%)	5 (0.5%)	<0.0001
Total	140 (100%)	67 (100%)	980 (100%)	335 (100%)	2804 (100%)	1377 (100%)	3161 (100%)	1617(100%)	<0.0001

* The p-value is for comparing the ratio of aerobic and anaerobic bacteria between the four groups using the Fisher's exact test. The test was performed for any and all types of bacteria

Table 4: The mortality rate in different groups of rats

Time			Survival		Total	P-value
			Died	Not died		
Control	Type	Control	0	5	5	-
		LO	0	5	5	
IO-24	Type	PIO	0	5	5	0.4444
		CIO	2	3	5	
		Total	2	13	15	
IO-48	Type	PIO	1	4	5	0.206
		CIO	4	1	5	
		Total	5	5	10	

Table 5: Culture results of the samples taken from the dead rats

Aerobic culture from peritoneal fluid	Anaerobic culture from peritoneal fluid	Blood culture in aerobic condition
300 CFU Gram Negative Rods (Pseudomonas and other Non-fermentive Gram Negative Rods) 1700 CFU Gram Positive cocci (Entroococci) 2000 CFU Gram negative bacilli (E. coli and other Entrobacteriacea) 100 CFU Gram Positive cocci (Staphylococci)	2100 CFU Gram negative bacilli (E. coli and other Entrobacteriacea) 5000 CFU Gram negative bacilli (Bacteroid) 1200 CFU Gram Negative Rods (Other Gram negative Rods) 1600 CFU Gram Positive cocci (Entroococci) 110 CFU Gram Positive cocci (Staphylococci)	Gram negative bacilli (E. coli and other Entrobacteriacea) Gram Positive cocci (Entroococci) Gram Positive cocci (Staphylococci)
400 CFU Gram Negative Rods (Pseudomonas and other Non fermentive Gram Negative Rods) 2100 CFU Gram Positive cocci (Entroococci) 3100 CFU Gram negative bacilli (E. coli and other Entrobacteriacea) 56 CFU Gram Positive cocci (Staphylococci)	3000 CFU Gram negative bacilli (E. coli and other Entrobacteriacea) 5000 CFU Gram negative bacilli (Bacteroid) 1000 CFU Gram Negative Rods (Other Gram negative Rods) 2000 CFU Gram Positive cocci (Entroococci) 68 CFU Gram Positive cocci (Staphylococci)	Gram negative bacilli (E. coli and other Entrobacteriacea) Gram Positive cocci (Entroococci) Gram Positive cocci (Staphylococci)

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Discussion

Bacterial translocation is the passage of toxic products and endotoxin from gastrointestinal tract to extra-intestinal sites such as the mesenteric lymph nodes, liver, spleen, kidney, and blood circulation [4].

Three mechanisms have been suggested for BT including [1] bacterial overgrowth and ecological disturbances of gastrointestinal system, [2] increased permeability in intestinal mucosal barrier, and [3] immune defense dysfunction [5, 6].

Both aerobic and anaerobic bacteria are usually found following intestinal obstruction. Our study indicated that with increased time of the obstruction, pattern of bacterial translocation changed from aerobic to anaerobic according to culture results in 24 and 48 hours after performing the obstruction. It was also found that E. coli was the most common type of bacteria in the IO-24 group, whereas enterococcus was the most common type of bacteria in the IO-48 group. The phenomenon of microbial synergy in these infections is well characterized.

It has been postulated that facultative organisms function in part to lower the oxidation-reduction potential in the microenvironment and that this change allows the propagation of obligate anaerobes [7]. Although further studies are needed to explain the reason for the change in the pattern of bacterial translocation, we believe that obstruction makes some changes in bacterial flora of the intestine in addition to tissue damage and partial ischemic and necrosis which facilitate growth of anaerobic bacteria. The predominance of some bacteria among clinical isolates suggests that they possess one or more factors that enhance their ability to cause disease. Typically, virulence factors associated with anaerobes confer the ability to evade host defenses, adhere to cell surfaces, produce toxins and/or enzymes, or display surface structures that contribute to pathogenic potential. Other studies have reported E. coli as the most common type of bacteria in aerobic group and enterococcus in the anaerobic group [8, 9].

On the other hand, it seems that in our study, increased number of bacteria in IO-24 compared to IO-48 group may be influenced by the condition that the immune system requires several hours to adjust bacterial translocation.

BT has been shown to occur in various patient populations ranging from patients with colorectal cancer, pancreatitis, intestinal obstruction, cholestasis, to those receiving parental nutrition and cases of malnutrition, with little evidence for the latter. It is also reported in elective surgical patients [6].

The intestinal epithelium changes related to obstruction are similar to those of the intestinal ischemia which include mitochondrial destruction, decreased capacity of the cell to produce energy and to preserve the equilibrium and structure of the mucosal epithelium; so, BT is also observed in ischemic patients [10, 11].

Our study showed similar BT in peritoneum and blood as well as after 24 and 48 hours of the obstruction. There was no difference between groups of partial and complete obstruction.

The role of immunological system in protecting the intestinal barrier function to avoid BT is known. Peyer's patches in the small intestine, along with lymphocytes, macrophages, and local IgA, develop an immune defense system [12].

In our study, BT was detected in all cases, and E. coli was the most common bacterium observed in both blood and peritoneal fluid. E. coli has also been frequently seen in other studies. Fernando et al. reported BT in 86% of the cases, while Berg et al. reported BT in 100% of the cases [13, 14].

In patients with the intestinal obstruction, the effect of manipulation and milking for decompression of the intestine has been evaluated. It is hypothesized that manipulation may cause paralytic ileus after surgery and may compromise the intestinal motility. Since, after resolving the obstruction and after 24 hours, the intestinal motilitybecomes normal, so in these patients, BT is not decreased with milking [15].

Systemic changes in patients with intestinal obstruction, that is associated with BT, may consequently result in the systemic inflammatory response syndrome. Therefore, these patients experience an elevated level of acute phase inflammatory markers such as the C-reactive protein (CRP) in the blood. Thus, CRP can be considered a predictor of vascular compromise during intestinal obstruction [16].

The relationship between bacterial translocation and survival rate has been confirmed by the increased rate of mortality following BT. Our study showed similar mortality rate following obstruction in all groups. No difference was observed between mortality rate of cases with partial or complete obstruction and that of groups of IO-24 or IO-48.

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Different methods are used to prevent BT and its systemic complications. Given the role of growth hormone (GH) in stimulating the mucus secretions, it is used as a drug in the prevention of BT. The protective effect of GH is related to the decreased rate of BT. Also, vitamin C and somatostatinanalogus are used in patients with BT, which lead to decreased rate of BT from 100% in the control group to 43% in the experimental group [17, 18].

Besides, myosin light chain kinase is used to avoid BT and protect intestinal mucosal barrier. In these cases, mucus histology including villous structure is maintained and the rate of mucosal damage such as villous blunting and epithelial sloughing is decreased. In addition, mucosal TNF (tumor necrosis factor) level is decreased and the rate of bacterial over growth and translocation is decreased [19].

Besides, saccharomyces cerevisiae strain UFMG 905 significantly protects the intestinal mucosal barrier. In case of using this material, measuring the level of interlukine-10 and IGA determines that the rate of immunological function is increased and thus BT is decreased. In addition, measuring the level of blood uptake of 99m TC-DTPA determines the rate of permeability [20].

Conclusions

In conclusion, bacterial translocation is an active process by which bacteria pass through the normally impermanent intestinal mucosal barrier and into lymph nodes or the systemic circulation. In this study, BT occurred both systemically (to the blood) and locally (to the peritoneum). No significant difference was noted between groups of partial and complete obstruction; however, pattern of bacterial translocation changed from aerobic to anaerobic with increased time of the obstruction.

Enterococcus was the most common type of bacteria in anaerobic group. It was found that in the IO-24 group, E. coli was the most common type of bacteria, whereas in the IO-48 group, the most common type of bacteria was enterococcus.

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