3 – ORIGINAL ARTICLE

Translocation of ^{99m}Tc labelled bacteria after intestinal ischemia and reperfusion[1]

Translocação de bactérias marcadas com ^{99m}Tc após isquemia e reperfusão intestinal

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ABSTRACT

PURPOSE: Ischemia and reperfusion of the small intestine disrupts gut barrier, causes bacterial translocation and activates inflammatory responses. An experimental study was planned to evaluate if ^{99m}Tc labelled *Escherichia coli* translocates to mesenteric lymph nodes, liver, spleen, lung and serum of rats submitted to mesenteric ischemia/reperfusion. Additionally, it was observed if the time of reperfusion influences the level of translocation.
METHODS: Forty male Wistar rats underwent 45 minutes of gut ischemia by occlusion of the superior mesenteric artery. The translocation of labelled bacteria to different organs and portal serum was determined in rats reperfused for 30 minutes, 24 hours, sham(S) and controls(C), using radioactivity count and colony forming units/g (CFU).
RESULTS: All the organs from rats observed for 24 hours after reperfusion had higher levels of radioactivity and positive cultures (CFU) than did the organs of rats reperfused for 30 minutes, C and S, except in the spleen ($p<0,01$).
CONCLUSION: The results of this study indicated that intestinal ischemia/reperfusion led to bacterial translocation, mostly after 24 hours of reperfusion.

Key Words: Bacterial translocation. Ischemia. Reperfusion. Intestine. ^{99m}Technecium labelled bacteria.

RESUMO

OBJETIVO: Isquemia e reperfusão do intestino delgado têm sido implicadas na quebra da barreira mucosa, na translocação bacteriana e na ativação de reações inflamatórias. Este estudo procurou avaliar se a *Escherichia coli* marcada com ^{99m}Tc transloca para linfonodos mesentéricos, fígado, baço, pulmão e soro de ratos submetidos a isquemia intestinal/reperfusão e se o tempo de reperfusão influencia o fenômeno.
MÉTODOS: Quarenta ratos Wistar foram submetidos a 45 minutos de isquemia intestinal através da oclusão da artéria mesentérica superior. A translocação de bactérias marcadas para

os diferentes órgãos e soro portal foi determinada em ratos após reperfusão mesentérica por 30 minutos, 24 horas, *sham* e controles, usando contagem de radioatividade e formação de unidades de colônias/grama de tecido (FUC/g).
RESULTADOS: Todos os órgãos dos animais observados com 24 horas de reperfusão intestinal tiveram níveis maiores de radioatividade e culturas positivas (FUC/g) do que os órgãos dos ratos reperfundidos por 30 minutos, os controles, e os sham, com exceção do baço ($p<0,01$).
CONCLUSÃO: Os resultados indicaram que a isquemia e reperfusão intestinal resulta em translocação bacteriana, mais intensamente após 24 horas de reperfusão.

Descritores: Translocação bacteriana. Isquemia. Reperfusão. Intestino. ^{99m}Tecnécio. Bactéria marcada.

Introduction

Bacterial translocation was originally defined and described by Berg and Garlington[1] as the passage of viable bacteria through the intestinal mucosa into the mesenteric lymph nodes (MLN) and to other tissues and organs. This situation, which occurs in some experimental and clinical conditions, is a problem of permanent concern to surgeons. Berg et al.[2] and Deitch et al.[3] popularized this concept and demonstrated the appearance of enteric bacteria in the MLN, liver, and spleen in a number of conditions including burns, endotoxemia and hemorrhagic shock. The methods by which bacterial translocation has been measured in laboratory animals have included quantitation of live bacteria by culture from organs and tissues out of intestines, demonstration of bacterial colonies in histopatological exams and measurement of radioactivity[4] in various organs after enteral administration of ^{14}C, ^{131}I labelled bacteria. The detection of bacterial DNA, which utilizes polymerase chain reaction, has also been used[5].
The present experiment was designed to analyze the effect of bowel ischemia, with and without reperfusion, on translocation of ^{99m}Tc labelled bacteria from the intestinal mucosa to MLN, liver, spleen, lung and serum. Additionally, it was observed if the time of reperfusion influences the level of translocation. As previously demonstrated, it is possible to label *Escherichia coli (E. coli)* efficiently with ^{99m}Tc [6].

Methods

E. coli were labelled with ^{99m}Tc, as follows. Briefly, a sample (0.1 mL) of *E. coli* ATCC-10536 culture, grown overnight in soybean casein medium, was incubated in 10mL of the same medium, under aeration, for 4 h at 37ºC. After that, different amounts of stannous chloride were added to 2 mL of the medium to reach final concentrations of 40, 130, 290, 400 and 580 ?M, respectively. The samples were then incubated at 37ºC for 10, 20, 40 and 60 min. After incubation, 37.0 MBq of ^{99m}Tc were added to each preparation and kept at 37ºC for 10 min. The tubes were then centrifuged at 3000x g for 25 min, washed and resuspended with normal saline. After three washes with saline, the ^{99m}Tc *E. coli* were incubated at 37ºC for 36h. Aliquots (100 ?L) of supernatant and resuspended precipitate in saline were withdrawn for determination of radioactivity. This procedure was repeated three times. In order to evaluate the bacterial viability, aliquots were taken from the last suspension, spread into a solid culture medium and incubated at 37ºC for 24 h. The effect of the procedure on the bacterial viability was assessed by comparing the colony-forming units per mL (CFU/mL) of labelled and unlabelled *E. coli*.

Forty male Wistar rats weighing 285?14g were maintained under conditions with controlled temperature, on a 12h light-dark cycle and fed *ad libitum* with commercially available rat chow and water. They were randomly divided into four groups (n=10 each), and named, respectively: C group, for non-operated rats, which were the controls, S group, for sham-operated, 30mAI group, for rats submitted to thirty minutes of intestinal reperfusion after ischemia, and 24hAI group, for those submitted to twenty four hours of reperfusion. All the animals were gavaged with ^{99m}Tc *E. coli*, four hours before the operative procedures started. After fasting overnight, all animals were anesthetized with intramuscular ketamine (50mg/kg) and pentobarbital (20mg/kg). For the two later groups, after laparotomy, the superior mesenteric artery (SMA) was occluded with an atraumatic arterial clip for 45 minutes.

At the end of the procedures, under aseptic conditions, a midline laparotomy was performed and blood was collected from the portal vein for culture and counting. One mL of serum was aliquoted for radioactivity counting. One gram of MLN complex, spleen, liver and lung were removed for counting and culture, if 1g of tissue was available; otherwise, the entire organ was weighed. Tissues were homogenized and solubilized. Aliquots of 0.2mL were processed and were then counted in an ANSR Abbott Scintillation Counter. Other portions (0,2mL) were cultured on selective MacConkey's agar and blood agar for detection of gram-negative and gram-positive bacteria, respectively. The plates were examined after 24 and 48 hours of incubation at 37^{o}C.

Procedures involving animals and their care were conducted in conformity with the *Guide for the Care and Use of Laboratory Animals,* US National Research Council, 1996. The data analysis were performed on a Compaq PC computer using the BioStat 2.0 program. The results were tabulated and compared by ANOVA using post hoc analysis with Newman-Keuls test. P<=0,05 was considered statistically significant.

Results

All animals survived the experimental protocol. When the 30mAI group and S group were compared, a significant variation on the labelled bacteria migration to different organs was found, except in the spleen. As shown in Table 1, the concentration of radio labelled *E. coli* was the greatest in the lung and liver in 24hAI rats. The S rats had the counts significantly lower than controls (p<0,01), except on the liver. The MLN, liver, lung and serum from 24hAI rats had significantly higher levels of radioactivity than did the organs from the 30mAI rats.

TABLE 1 - Level of radioactivity (mean counts per minute per gram) from MLN, Spleen, Liver, Lung and Serum after ^{99m}Tc *E. coli* translocation studies.

Groups	n	MLN	Spleen	Liver	Lung	Serum
C	10	50?18.5	6?1.5	57?14.9	85?29	38?14
S	10	47?9	5?1.2	71?17	76?12	29?8
30mAI	10	88?23 *	0.0	682?113 *	795?132*	120?27*
24hAI	10	155?54 †	4.0?0.8	2137?331†	1037?119†	222?57 †

C, Control; S, Sham; 30mAI, thirty minute of reperfusion after ischemia; 24hAI, 24 hours of reperfusion after ischemia.
* p < 0,01 compared to sham, control and 24hAI
† p< 0,01 compared to control, sham and 30mAI

TABLE 2 - Magnitude (CFU per gram of tissue) of bacterial translocation to several organs and serum.

Groups	n	MLN	Spleen	Liver	Lung	Serum
C	10	5?1.7	0.0	2?1.8	3?2.3	2?1.8
S	10	4.2?2	2?0.2	3?2.2	5?2.7	0
30mAI	10	13?3.2*	2?1.2	28?8*	55?13.7*	3.5?2.6
24hAI	10	30?9.5 †	0.0	77?18.9 †	81?15.1 †	9?3.2 †

* p<0,01 compared to control, Sham and 24hAI
† p<0,01 compared to control, Sham and 30mAI

The level of positive cultures with colony-forming units (CFU) was significantly higher in 24hAI rats than in 30mAI, S and C ones (Table 2). The S group was the only one where the serum was free of any bacterial colony. As observed with the mean count of radioactivity in the spleen, bacteria were absent or rarely detected in the culture of this organ (Tables 1 and 2). The most common bacteria cultured from the organs were *E. coli and Stapylococcus.*

Discussion

The gastrointestinal tract is inhabited by a large number of bacteria species and has a high concentration of endotoxin[7]. The gut has efficient defense mechanisms to protect the host from being invaded by their own flora or their toxins. These include an ecological balance of intestinal micro flora, that prevents overgrowth and translocation of any particular species**,** and the physical barrier composed of the intact intestinal mucosa. In addition, immune defenses and other factors provide additional levels of protection[8]. The etiology of bacterial translocation from the gut may include: gut barrier failure by multiple mechanisms in bowel ischemia and reperfusion[9], impaired host immune defense, and disturbed ecology of intestinal flora[2].
The gut has been suggested to be a port of entry for bacteria after intestinal mucosal injury and endotoxin challenge[10]. The translocation process involves the initial attachment of the bacteria to the gut wall, which by itself can elicit production of cytokines and initiate the subsequent inflammatory response. Once intact microbes penetrate the mucosa, they may be transported to distant organs of even the systemic circulation[11].
As shown in the present study, bowel ischemia and reperfusion promoted bacteria translocation. In addition, when compared to the control and sham, this phenomenon was significantly higher for liver and lungs in all other groups. Redan et al.[12] speculate that the route of bacterial translocation is through lymphatics into the right side of the heart and then to the lung. The pulmonary vascular bed would then represents the first capillary system in

which the translocated bacteria encounter circulating phagocyte cells. In fact, the majority of colony-forming units of bacteria were found in the lung. In the spleen the bacteria were absent or rarely detected. On the other hand, twenty-four hours after mesenteric reperfusion, the liver exhibited the greatest mean count of radioactivity, as observed in Table 1. It was shown that an initial episode of ischemia/reperfusion causes an adaptative response, which protects the intestine against subsequent insult, and this protection is associated with increased mucus production[13]. In the present study, when it was compared the mean counts of radioactivity and the CFU of bacteria in 30mAI and 24hAI groups, a significant difference was observed. The bacterial translocation occurred after 30 minutes of ischemia/reperfusion, but it was significantly higher in the 24hAI group. Thus, hypoxia, followed by change in intestinal barrier function, generates a vicious cycle of increased permeability, leading to toxic mediators release, and resulting in a further increase in gut permeability. Therefore, facilitating the bacterial translocation[14]. However, no significant difference in radioactivity and CFU were found when it was compared the S group, where the intestines were gently manipulated, and the control (C group). Increased serum levels of pro-inflammatory cytokines have refleted the ischemia/reperfusion injury, as demonstrated by in vivo experimental trials[15,16,17].

Conclusion

In conclusion, ^{99m}Tc labelled bacteria were detected in serum, lungs, liver and MLN after mesenteric ischemia and reperfusion, and this likely represents translocation. So, the ^{99m}Tc labelled *E. coli* seems to be a viable model to study bacterial translocation. The fenomenon was more evident after 24 hours of reperfusion than after 30 minutes, meaning that the time is an important factor for translocation.

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