Dissemination of bacteria labeled with technetium-99m after laparotomy and abdominal insufflation with different co_2 pressures on rats[1]

Disseminação de bactérias marcadas com tecnécio-99m após laparotomia e insuflação com diferentes pressões de co_2 em ratos

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ABSTRACT

Purpose: To asses the dissemination of bacteria labeled with technetium-99m (^{99m}Tc) from peritoneal cavity after different surgical procedures. **Methods**: Bacteria of the *Escherichia coli* species labeled with ^{99m}Tc were used in a concentration of 10^8 units of colony-makers for ml (UFC/ml) and 1ml was inoculated through intra-peritoneal via. Forty-eight rats were divided into four groups: control, laparotomy, pneumoperitoneum with 10mmHg and pneumoperitoneum with 20mmHg of CO_2. Procedures were performed 20 min after injection of the inoculum and lasted 30 min. Animals were sacrificed after six hours (Group 1) and 24 hours (Group 2). Samples of blood, liver and spleen were collected for radioactivity counting. **Results**: After six hours, indirect detection of the bacteria in different organs was uniform in all groups. After 24 hours, a larger detection of technetium was observed in the livers of animals of the group insufflated with 20mmHg of CO_2, when compared with those of control group (p<0.01). The other groups did not present statistically significant variations. **Conclusions**: The use of a higher intra-abdominal pressure was associated with a higher bacterial dissemination to the liver. The application of lower intra-abdominal pressures may be associated with a lower dissemination of the infectious status during laparoscopic approach of peritonitis status.

Key words: Peritonitis. Pneumoperitoneum. Escherichia coli. Sodium Pertechnetate Tc 99m. Rats, Wistar.

RESUMO

Objetivo: Avaliar a disseminação de bactérias marcadas com tecnécio-99m (^{99m}Tc) a partir da cavidade peritoneal após diferentes procedimentos cirúrgicos. **Métodos**: Foram utilizadas bactérias da espécie *Escherichia coli* marcadas com ^{99m}Tc em uma concentração de10^8 unidades formadoras de colônia por ml (UFC/ml) sendo inoculado 1ml por via intra-peritoneal. Quarenta e oito ratos foram divididos em quatro grupos: controle, laparotomia, pneumoperitôneo com 10 mmHg e pneumoperitôneo com 20 mmHg de CO_2. Os procedimentos foram realizados 20 minutos após a injeção do inóculo e duraram 30 minutos. Os animais foram sacrificados após seis horas (grupo 1) e 24 horas (grupo 2). Foram coletadas amostras de sangue, fígado e baço para contagem radioativa. **Resultados**: Após seis horas, a detecção indireta das bactérias nos diferentes órgãos foi uniforme em todos os grupos. Após 24 horas, observou-se uma maior detecção de tecnécio nos fígados dos animais do grupo insuflado com 20 mmHg de CO_2, quando comparados aos do grupo controle (p<0,01). Os outros grupos não apresentaram variações estatisticamente significativas. **Conclusões**: A utilização de pressões intra-abdominais mais elevadas associou-se a uma maior disseminação bacteriana para o fígado. A utilização de pressões intra-abdominais menos elevadas na abordagem da peritonite pode estar associada a uma menor disseminação do quadro infeccioso.

Descritores: Peritonite. Pneumoperitônio. Escherichia coli. Pertecnetato Tc 99m de Sódio. Ratos, Wistar.

1. Research performed at the Laboratory of Experimental Surgery, Medical Sciences School, State University of Rio de Janeiro (UERJ), Brazil.

Introduction

Since the 80s, with the advent of videosurgery, laparoscopic access to peritoneal cavity began to play a very increasing role in the treatment of intra-abdominal affections. The success accomplished with the treatment of biliary vesicular lithiasis paved the way to videolaparoscopy use in divers situations. The good results achieved in gastroenterological, splenic, endocrinal, gynecological and of urgency surgeries have already been largely described.[1,2,3] Laparoscopic surgery is associated with less pain in the postoperative period and a more precocious return to normal activities when compared with conventional surgery. Nonetheless, the use of laparoscopy in a septical environment and its consequences in a local and systemic level have been the target of a large discussion. The mechanism by which intraperitoneal bacteria have disseminated to the blood current has not yet been clarified.

In diffuse bacterial peritonitis status, the pneumoperitoneum produced through CO_2 insufflation may increase the risk of bacterial dissemination, bacteremia and sepsis.[4,5] This phenomenon has been assessed through experimental models of peritonitis. Nonetheless, results of the researches in this area have been controversial.[4,5,6] Ozmen et al.[5] demonstrated, in one model of abdominal sepsis (intraperitoneal infusion of *Escherichia coli* (*E. coli)* colonies in rabbits), a higher risk of bacterial dissemination for organs at a distance in the group submitted to laparoscopy when compared with laparotomized animals. However, in a recent study, we demonstrated a less number of peritoneal cultures and positive hemocultures in mice submitted to cavitary insufflation when compared with animals submitted to laparotomy after intraperitoneal infusion of 0.5mL of a solution containing 1 X 10^8 CFU/mL of *E. coli*. [7] Other authors did not observe significant alterations in animals that are carriers of peritonitis submitted to laparotomy or cavitary insufflation with CO_2 in relation to bacteremia, endotoxemia, and physiological alterations secondary to sepsis.[8,9]

Several models have been used in the attempt of mimetizing the conditions observed in human peritonitis and sepsis, however, extrapolation of experimental results for clinical practice has been, most of the times, disappointing.[10]

An important challenge nowadays is the development of new models for the study of mechanisms of bacterial dissemination secondary to peritonitis and the impact of several surgical procedures facing abdominal sepsis status.

Within this philosophy, a new experimental model was developed through the use of bacteria labeled with a radioactive isotope (technetium-99m) for assessing bacterial dissemination from peritoneal cavity in rats submitted to laparotomy and peritoneal insufflation with different CO_2 pressures.

Methods

We used a model with rats submitted to laparotomy and pneumoperitoneum produced by insufflation with different CO_2 pressures. We conducted the intra-peritoneal inoculation of suspension of *E. coli* labeled with technetium-99m. Uptake of this bacterium was assessed through the blood, spleen, and liver of the animals in two different times after inoculation.

The procedures were performed at Laboratory of Experimental Surgery of the Medical Sciences School of the Universidade do Estado do Rio de Janeiro/ UERJ in collaboration with the Laboratory of Experimental Radiopharmacy of the Department of Biophysics and Biometry of Instituto de Biologia Roberto Alcântara Gomes - IBRAG/UERJ. This work was approved by the Ethics Committee on Animal Research of the Laboratory of Experimental Surgery of the Medical Sciences School of UERJ.

Labeling and inoculation of bacteria

We used bacteria of the species *E. coli* of the lineage AB1157, obtained in the Laboratory of Experimental Radiopharmacy of IBRAG. Bacteria were labeled with technetium-99m under the form of sodium pertechnetate through a reaction with stannous chloride ($SnCl_2.2H_2O$) which acts as a reductor agent for technetium-99m.[11]

A sample of *E. coli* was added to a proper culture medium and incubated from 15 to 18 hours (overnight) at a temperature of 37ºC. From this culture, 200µl were withdrawn, put in a culture medium and incubated in agitator for more two hours under the same temperature (reculture). From reculture, we withdrew an aliquot which was homogenized and centrifuged at 4000 rotations per minute (rpm) for 25 min. We despised the supernatant and added SF0.9% to the precipitate, which was resuspended and centrifuged successive times until we obtained a suspension of bacteria free from culture medium. This suspension was put in a vacuum tube, and we added stannous chloride as reductor agent of technetium-99m. This solution was diluted, homogenized and then incubated in agitator for 15 min at 37ºC. Then technetium-99m was added, followed by homogenization, new incubation in the agitator for 10 minutes at 37ºC, and posterior centrifugation at 4000rpm for 25 min. Supernatant and the resuspended precipate were taken for radioactive counting, and a percentile of labeling of bacteria superior to 95% was obtained. [12]

A final concentration of bacteria labeled with technetium-99m was obtained corresponding to 10^8 units colony-makers per ml (UFC/ml). The inoculated volume was 1ml per animal through intraperitoneal via.

Animals and procedures

Male Wistar rats (*Rattus norvegicus)* weighing 270 — 362g were used in this work. Animals were kept in a room with controlled temperature (25° C) and a 12 hours light/ dark cycle. Animals anesthetized with intraperitoneal sodium thiopental (40mg/Kg) received a 1.0 mL intraperitoneal injection of a solution containing 1 x 10^8 CFU/mL of *E. coli.* Animals were divided into groups of 12 animals according to the type of access to peritoneal cavity as following: Control — absence of any further procedure; Laparotomy

— animals suffered a 2.5cm anterior median abdominal incision; CO_2 10 — animals suffered an anterior abdominal puncture with a 20 gauge needle followed by peritoneal insufflation with CO_2, maintaining a 10mmHg intracavity pressure (KARL STORZ ENDOSKOPE - ELETRONIC ENDOFLATOR, model 26430520); CO_2 20 — animals suffered an anterior abdominal puncture with a 20 gauge needle followed by peritoneal insufflation with CO_2, maintaining a 20mmHg intracavity pressure. The procedures described were performed 20 min after the injection of bacterial solution.

Each of the four groups was subdivided into two subgroups according to the time elapsed after bacteria inoculation: Group 1 — sacrificed after six hours of bacterial inoculation; Group 2 — sacrificed after 24 hours of bacterial inoculation. Animals died from a lethal dose of thiopental.

Tissue withdrawal for uptake study

Animals were submitted to thoracolaparotomy and 1ml of intra-cardiac blood was aspirated, and liver and spleen were withdrawn and weighed in a precision scale. Liver and spleen fragments were then withdrawn, rightly weighed, and as well as the blood were put in tubes appropriate for radioactivity counting.

Percentile calculation of bacteria uptake

Percentile calculation of bacteria uptake used a dose standard containing the same volume and the same activity of the *E. coli* suspension labeled with technetium-99m inoculated in animals. The standard counting was considered as 100% of radioactivity inoculated in animals. The uptake percentile of each sample was calculated using the formula:

$$\bullet\ \%\ \text{of uptake} = \frac{\text{Sample counting per minute x 100}}{\text{Counting per standard minute}}$$

Taking into consideration the liver and spleen mass, the uptake percentile per gram of tissue was calculated by the formula:

$$\bullet\ \%\ \text{of uptake/g} = \frac{\%\ \text{of each tissue uptake}}{\text{Mass of each sample (g)}}$$

The uptake percentile of each organ (total mass) was calculated by the formula:

% of organ uptake = % of uptake/g x organ total mass (g)

Radioactivity determination

The tubes containing fragments of the withdrawn organs and blood were put in appropriate shelves, and radioactivity counting was conducted in a gamma ray-scintillometer.

Statistical analysis

Results were represented as mean and standard error of the mean (SEM) and statistically assessed through Newman-Keuls-Student test, and values considered significant were $p \leq 0.05$.

Results

Uptake in blood samples

After six hours, technetium-99m uptake in the blood of the group laparotomized and the group insufflated with 10mmHg of CO_2 presented a mean value discreetly higher, followed by animals of the control group and the group insufflated with 20mmHg of CO_2 pressure. There was no statistical significance between the groups. In the 24 hour-assessment, the group insufflated with 20mmHg of CO_2 pressure began to present a more elevated technetium-99m mean uptake, followed by the laparotomy and insufflation groups with 10mmHg of CO_2 pressure. Control group presented a less elevated technetium-99m uptake than the other groups; nonetheless differences were not statistically significant (Figure 1).

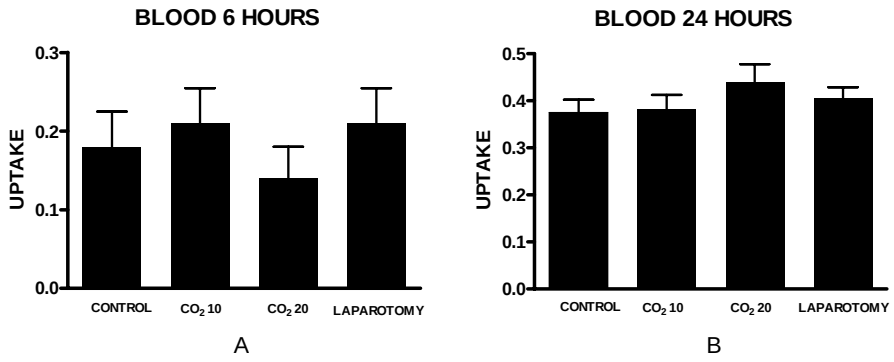

FIGURE 1 - Technetium-99m uptake in the blood after six hours (A) and 24 hours (B). Each bar represents the mean ± SEM of six animals. There were no statistically significant variations between the groups

Uptake in spleen samples

In the six hour-analysis, technetium-99m uptake in the spleen of the group insufflated with 20mmHg of CO_2 presented a mean value discreetly higher, followed by animals of the group insufflated with 10mmHg of CO_2 pressure, the laparotomized group and the control group. There was no statistically significance between the groups. In the second period (24 hours), the group insufflated with 20mmHg of CO_2 pressure maintained a more elevated technetium-99m mean uptake, followed by the groups of laparotomy and of insufflation with 10mmHg of CO_2 pressure. Control group began to present a less elevated technetium-99m mean uptake than the other groups; however there was no statistically significant relationship (Figure 2).

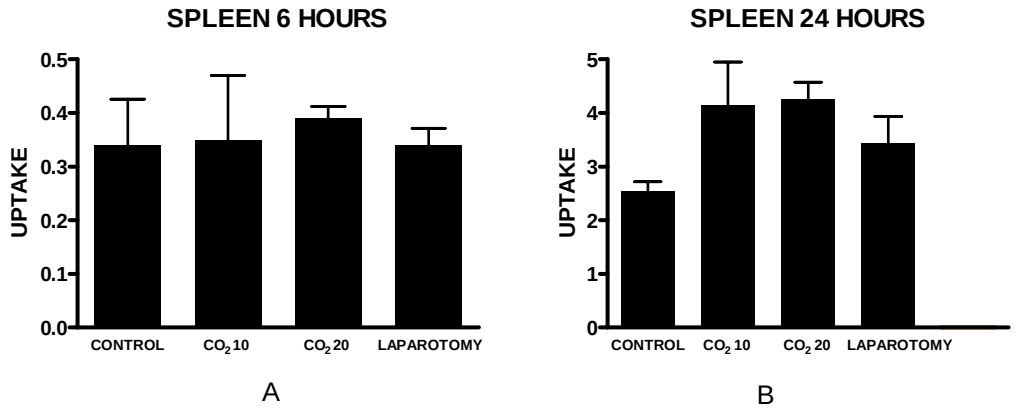

FIGURE 2 - Technetium-99m uptake in the blood after six hours (A) and 24 hours (B). Each bar represents the mean ± SEM of six animals. There were no statistically significant variations between the groups

Uptake in liver samples

After six hours, we observed a technetium-99m uptake in the liver of the laparotomized group with a more elevated mean value, followed by animals of the group insufflated with 20mmHg of CO_2, of the group insufflated with 10mmHg of CO_2 pressure, and of control group. There was no statistical significance between the groups. After 24 hours, the group insufflated with 20mmHg of CO_2 pressure began to present a more elevated technetium-99m mean uptake, with a statistically significant difference in relation to control group (p<0.01). The groups of laparotomy and of insufflation with 10mmHg of CO_2 pressure did not present a significant statistical relationship (Figure 3).

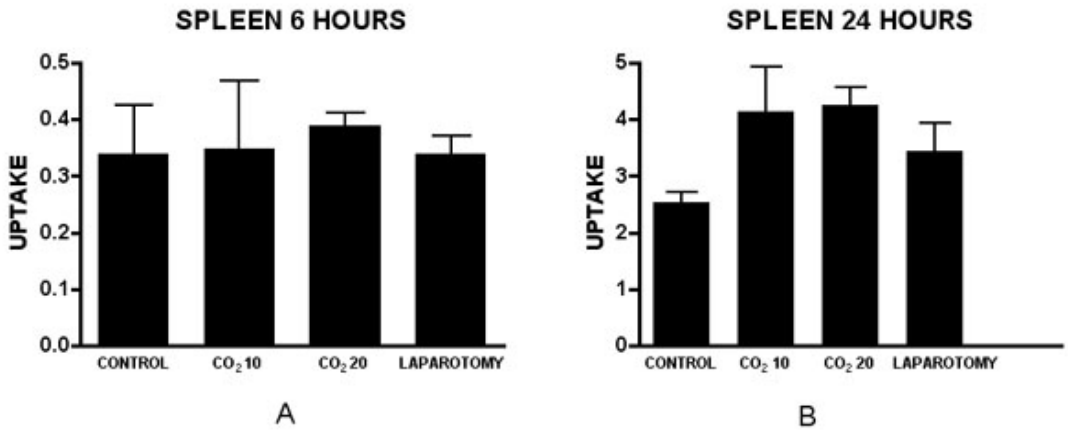

FIGURE 3 - Technetium-99m uptake in the liver after six hours (A) and 24 hours (B). Each bar represents the mean ± SEM of six animals. * p<0.01 vs control

Discussion

Surgical treatment is a fundamental part of the therapeutic approach of bacterial peritonitis status. Drainage of a septical focus, resection of perforated or necrosed organs, and abundant peritoneal irrigation associated with the adequate clinical treatment represent the best way to solve an intra-abdominal infection. In order to offer the best therapeutical options, it is important to know the impact of surgical trauma on the organism response to pathogenical microorganisms.

Pneumoperitoneum effects on immune response, bacterial dissemination and sepsis development have been extensively studied [7,13] with the increasing use of the laparoscopic method in statuses associated with peritonitis.

A large variety of animal models have been proposed aiming to analyze many aspects of the laparoscopic approach in the status of peritonitis and sepsis such as: type of gas and different levels of pressure employed in peritoneal insufflation, [14,15] duration time of pneumoperitoneum, bacterial species inoculated and animal species studied, inoculation via, as well assessment methods.[16,17]

Despite the fact that polymicrobial models such as cecal ligation and puncture (CLP) [18] and the intra-peritoneal injection of feces [19] produce a peritonitis status similar to that found in clinical practice where polomicrobial infection prevails, quantification of microorganism dissemination may be complex since the animals may be infected by different bacterial species.

Models that use intraperitoneal infusion of a pre-determinate bacterial inoculum are more adequate when what is intended is to analyze the peritoneal clearance of bacteria and its growth in several organic spaces. Besides, they cause a more uniform organic response, which facilitates the analysis of the response to a determinate substance or to surgical intervention. [7]

Among several radiopharmaceuticals used for labeling of biological structures, technetium-99m stands out for its physical chemical characteristics such as high availability, short midlife time, gamma radiation emission, [20] and for allowing a labeling method relatively simple, effective, fast, of easy assessment, and of low cost. [11]

Studies using bacteria labeled with technetium-99m have already been conducted in order to assess pulmonary bacterial depuration [21] and bacterial translocation in statuses of hemorrhagic shock [20] and in obstructive ictericia. [22]

In experimental models of peritonitis, bacterial dissemination at distance has been assessed predominantly through microbiological analysis, cellular countings and dosages of pro-inflammatory cytokines. [7,23] The use of *E. coli* labeled with technetium-99m in a model comparing the pneumoperitoneum with laparotomy allows the introduction of a new method of study for bacterial dissemination assessment in peritonitis statuses.

Among the parameters used in this work, 10mmHg and 20mmHg of CO_2 pressures were chosen with the aim of making more evident bacterial dissemination degrees related to different pressures. The same reasoning is applied to the choice of assessment times of six and 24 hours after bacterial inoculation. Different levels of pneumoperitoneum pressure and the time elapsed after bacteria inoculation seem to associate directly with the extension and severity of peritonitis.[14,16]

The higher uptake in the group insufflated with 20mmHg may be related to the peritoneal reaction to intra-peritoneal pressure increase. Volz et al. [24] experimentally demonstrated, through electronic microscopy, that pneumoperitoneum with CO_2 leads to contraction of mesothelial cells causing an opening between intercellular junctions. This phenomenon may facilitate the passage of microorganisms. Besides, the increased intra-abdominal pressure causes an increase of pressure gradient, which associates with the diaphragmatic distension and contributes to bacterial dissemination. [25]

On the other hand, the difference in the uptake observed only in the late group alerts to a discerning assessment of laparoscopy use in peritonitis status with many hours of evolution.

The higher bacteria uptake in the liver may be a consequence of the important role of this organ in the mononuclear phagocytic system. Hepatic detection of bacteria labeled with technetium-99m significantly higher in the group submitted to pneumoperitoneum produced by insufflation with 20mmHg of CO_2 suggests that elevated intra-abdominal pressures may be associated with a higher bacterial dissemination at distance. This datum may have clinical relevance in the surgical approach of bacterial peritonitis status pointing to the need of using less elevated intra-abdominal pressures in this situation.

New studies with the use of labeled bacteria associated with already well established peritonitis models may help to understand the peritoneal dynamics secondary to peritonitis and the phenomena of bacterial dissemination.

Conclusions

The use of a higher intra-abdominal pressure was associated with a higher bacterial dissemination to the liver. The application of lower intra-abdominal pressures may be associated with a lower dissemination of the infectious status during laparoscopic approach of peritonitis status.

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Conflict of interest: none
Financial source: Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)

Received: August 10, 2007
Review: October 15, 2007
Accepted: November 12, 2007

How to cite this article
Pitombo MB, Faria CASA, Bernardo LC, Steinbruck K, Bernardo Filho M. Dissemination of bacteria labeled with technetium-99m after laparotomy and abdominal insufflation with different Co_2 pressures on rats. Acta Cir Bras. [serial on the Internet] 2008 Jan-Feb;23(1). Available from URL: http://www.scielo.br/acb