

## Histological features of peritoneal lavage with ropivacaine in rats with fecal peritonitis<sup>1</sup>

### Características histológicas da lavagem peritoneal com ropivacaína em ratos com peritonite fecal

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#### ABSTRACT

**PURPOSE:** To evaluate the histological features in lungs, peritoneum and liver of rats subjected to fecal peritonitis and treated with peritoneal lavage with 0.2% ropivacaine.

**METHODS:** Twenty Wistar rats were subjected to laparotomy 6 h after the fecal peritonitis induction with autogenous stool. Rats were randomly distributed into 4 groups: I - (n=5) Control, no treatment; II - (n=5) Drying of the abdominal cavity; III - (n=5) Abdominal cavity lavage with 3 ml 0.9% saline solution and drying; and IV - (n=5) Abdominal cavity lavage with 3 ml 0.2% ropivacaine and drying. The animals that died underwent necropsy, and the surviving ones were subjected to euthanasia on the 11th day post-surgery. Fragments of liver, lungs and peritoneum were removed for histological evaluation.

**RESULTS:** The animals that received peritoneal lavage (groups III and IV) showed greater survival than the drying and control groups. Lavage with ropivacaine prevented death during the observed period. Peritoneal lavage with ropivacaine maintained the architecture of the lung, peritoneum and liver without any important histological alterations. The histopathological findings analyzed correlated with greater survival of group IV. **CONCLUSION:** Treatment of fecal peritonitis in rats with peritoneal lavage using 0.2% ropivacaine demonstrated a reduction in histopathological alterations related to inflammatory response and sepsis.

**Key words:** Peritonitis. Peritoneal Lavage. Anesthetics. Sepsis. Rats.

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#### RESUMO

**OBJETIVO:** Avaliar os aspectos histopatológicos em pulmões, peritônios e fígados de ratos submetidos à peritonite fecal e tratados com lavagem peritoneal com ropivacaína a 0,2%.

**MÉTODOS:** Utilizou-se 20 ratos Wistar, submetidos à laparotomia 6 horas após a indução de peritonite fecal com fezes autógenas, distribuídos aleatoriamente em quatro grupos: I- (n=5) Controle, nenhum tratamento; II- (n=5) Enxugamento da cavidade abdominal; III- (n=5) Lavagem da cavidade abdominal com 3 ml de solução salina 0,9% e enxugamento; IV- (n=5) Lavagem da cavidade abdominal com 3 ml de ropivacaína a 0,2% e enxugamento. Os animais que morreram foram necropsiados e os sobreviventes foram eutanasiados no 11º dia do pós-operatório. Retirou-se fragmentos do fígado, pulmões e do peritônio dos animais para estudo histopatológico.

**RESULTADOS:** Os animais que receberam lavagem peritoneal (grupos III e IV) apresentaram maior sobrevida que os grupos enxugamento e controle. A lavagem com ropivacaína impediu o óbito no período avaliado. A lavagem peritoneal com ropivacaína manteve a arquitetura do pulmão, peritônio e fígado sem alterações histológicas importantes. Os achados histopatológicos analisados foram condizentes com o maior tempo de sobrevida no grupo IV.

**CONCLUSÃO:** A lavagem peritoneal com ropivacaína a 0,2% no tratamento da peritonite fecal em ratos demonstrou reduzir as alterações histopatológicas relacionados à resposta inflamatória e sepse.

**Descritores:** Peritonite. Lavagem Peritoneal. Anestésicos. Sepse. Ratos.

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## Introduction

The mortality and incidence rates of sepsis have increased in the last few years. In the United States, it is estimated that there is a yearly incidence of 750,000 septic patients with 28.6% mortality, which represents a cost US \$16.7 billion dollars in healthcare<sup>1</sup>. Peritonitis is one of the most important causes of sepsis and death in operating rooms and intensive care units.

In peritonitis, sepsis occurs when an infectious intra-abdominal focus unleashes a systemic inflammatory response. Such a response is characterized by the activation of several systems, including complement, coagulation, kinins and fibrinolysis, cells (endothelial, leucocytes, monocytes, macrophages and mastocytes), and the release of mediators (free oxygen radicals, histamine, eicosanoids, coagulation factors, and cytokines)<sup>2,3</sup>.

The classic treatment of peritonitis is the mechanical removal of the contaminants, restoration of anatomical integrity, and systemic administration of antimicrobial drugs. The indiscriminate use of antimicrobials has contributed to the development of resistance in several strains of microorganisms. In 1946 in the United States, only 5% of staphylococci isolated from American hospitals were penicillin-resistant. In 1949, 1950, and 1959, the penicillin resistance of hospital cultures was described as 29, 50, and 80%, respectively. Currently, more than 80% of *S. aureus* isolated from hospitalized patients in Brazil and approximately 70% of cultures isolated from patients in the community are resistant to the natural penicillins.<sup>4</sup>

The increasing incidence of bacterial resistance associated with the difficulty of developing new antibiotics has directed studies in the use of alternative techniques in the treatment of peritonitis. Several studies investigate the modulation of the inflammatory response with the goal of increasing survival and reducing sepsis mortality. Several publications reported a broad range of anti-inflammatory actions of local anesthetics through their effects on the cells of the immune system as well as platelets, erythrocytes, and the microorganism itself<sup>5</sup>. Indeed, such agents have been utilized in the treatment of several conditions linked to inflammatory processes like interstitial cystitis, ulcerative proctitis, arthritis, herpetic infections and burns<sup>6</sup>.

The anti-inflammatory mechanism of local anesthetic action is not completely understood, but it seems to encompass a reversible interaction with the proteins and lipids of the plasma membrane as well as the regulation of metabolic cellular activity, migration, exocytosis, and phagocytosis. Thus, in our present study, we investigated the histological characteristics of liver, lung, and peritoneum fragments of rats subjected to fecal

peritonitis stimulated by autogenous feces and treated with lavage of the abdominal cavity with 0.2% ropivacaine.

## Methods

This study was approved by the Ethics in Research Committee of the School of Medicine of the Federal University of Minas Gerais State (UFMG) and was assigned protocol number 028/09 (COEP-CETEA). Twenty male Wistar rats originating from the EMESCAM Research Center ranged in weight between 280 and 320 g were operated on and randomly distributed into four groups of five animals each.

These animals were housed in cages of five animals each under constant environmental conditions and were fed an appropriate diet (Nuvital<sup>®</sup>) and water *ad libitum*. Animals were allowed a period of seven days of acclimatization before the experiment was initiated.

The animals were anesthetized by intramuscular injection of (S+) ketamine chlorhydrate (Cristalia<sup>®</sup>, Sao Paulo, Brazil) at a dose of 10 mg/kg animal weight<sup>7</sup> in the anterior aspect of the right leg. A 16G teflon catheter was subsequently placed in the left inferior abdominal quadrant. Next, a suspension of autogenous fecal material, prepared by dilution of 2 g freshly passed feces in 17 ml saline solution, was injected into the abdominal cavity. Prior to the injection, the suspension was filtered through gauze to allow free flow through the needle. Of this suspension, 10 mL/kg animal weight was injected into the abdominal cavity<sup>7</sup>.

Six hours after the peritonitis induction, the rats were anesthetized with a mixture of xylazine chlorhydrate (10 mg/kg; Lab. König, SA<sup>®</sup>, Argentina) and (S+) ketamine chlorhydrate (50 mg/kg; Cristalia<sup>®</sup>, Sao Paulo, Brazil) and subjected to median laparotomy with an incision of approximately 2 cm in length, after which abdominal cavity examination was performed.

The animals were divided in the following groups:

Group I - (n=5) Control, no treatment; Group II - (n=5) tender drying of the contents of the abdominal cavity with dry gauze; Group III - (n=5) abdominal cavity lavage with 3 mL 0.9% saline solution and drying; and Group IV - (n=5) abdominal cavity lavage with 3 mL 0.2% ropivacaine and drying. In groups III and IV, saline solution (group III) or 0.2% ropivacaine (group IV) was injected in the cavity after drying of the abdominal cavity with dry gauze and left there for 3 minutes. During this time, the solution was carefully handled between the abdominal viscera to allow for greater contact with the peritoneum. After this procedure, the peritoneal fluid was gently dried off with dry gauze to remove the maximum amount of fluid. The abdominal wall was stitched in a

single plane using mononylon (4-0).

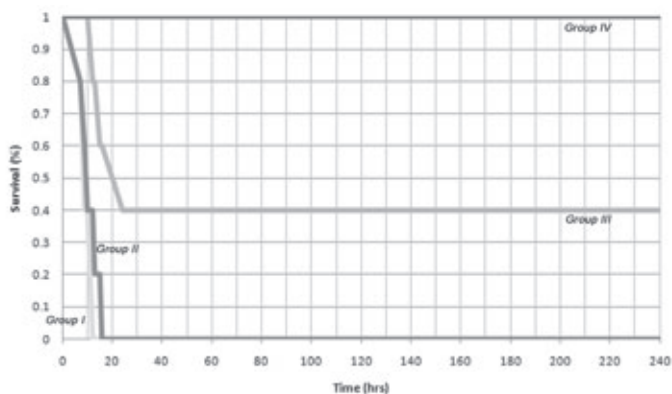
Hydration was performed with one subcutaneous 5-mL injection of 0.9% saline solution every 24 h for two days. Analgesia was performed with subcutaneous injection of nalbuphine chlorhydrate (0.3 mg/kg animal weight) every 8 h for 2 days.

The animals that died underwent necropsy, and those that survived were euthanatized on the 11<sup>th</sup> day post-surgery with an intra-peritoneal injection of 50 mg/kg pentobarbital.

Besides survival time, liver, lung, and peritoneum fragments of the dead and euthanized animals were evaluated by histopathological studies. Collected material was fixed in 10% formaldehyde and stored in a volume of solution corresponding to 10 times the volume of the sample. Material was prepared in a Lupe<sup>®</sup> processor with 11 washes, embedded in paraffin blocks, and cut with a microtome (SLE<sup>®</sup>) at 2.5 microns followed by hematoxylin/eosin staining.

**Results**

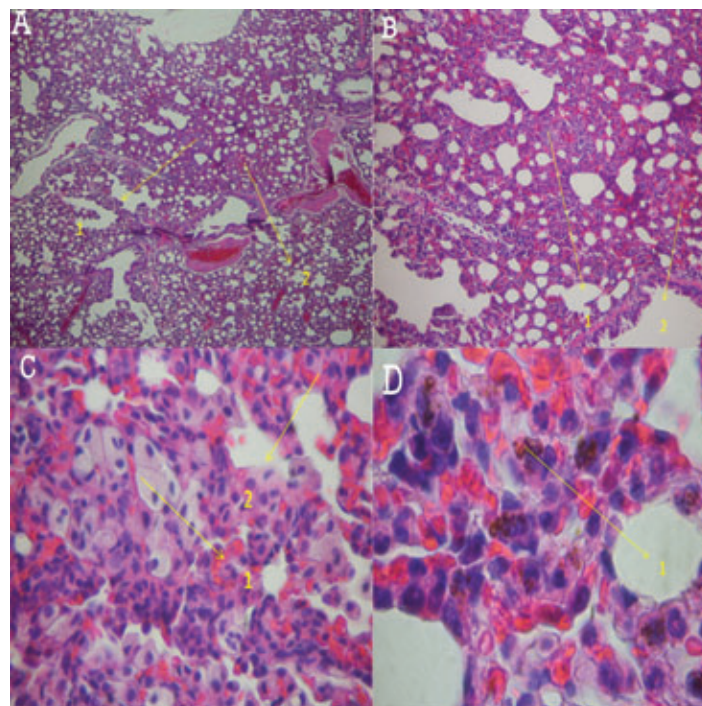
Figure 1 shows that the survival of animals when peritoneal lavage was performed was greater than that of the control and drying groups. The survival curve shows a mortality of 100% in 12 hours for the animals in group I and 16 hours for the animals in group II. Group III presented 60% mortality in 24 hours. Ropivacaine lavage prevented death until the last day of monitoring in all animals in this group.



**FIGURE 1** - Survival curve after laparotomy for peritonitis treatment.

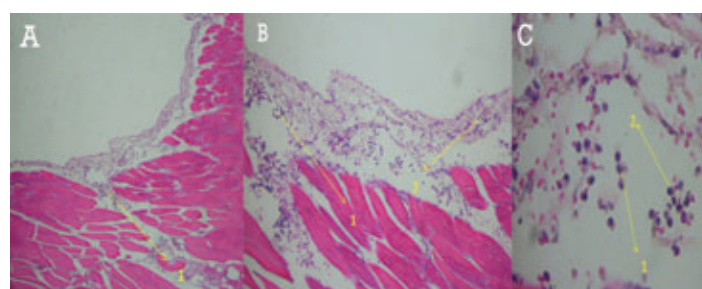
The lung fragments of the rats that did not receive treatment (Group I - control) presented with diffuse bronchopneumonia, filling of the alveolar spaces by macrophages and monocytes, diffuse alveolar hemorrhage, and thickening of alveolar septa (Figure 2A). The presence of an intense lymphohistoplasmacytic and neutrophilic exudate in the interior of the respiratory bronchioli (Figure 2B) was also observed. Macrophage foam cells

and neutrophils were present within alveolar spaces (Figure 2C). In addition, several hemosiderophages were visualized in the lung parenchyma (Figure 2D).



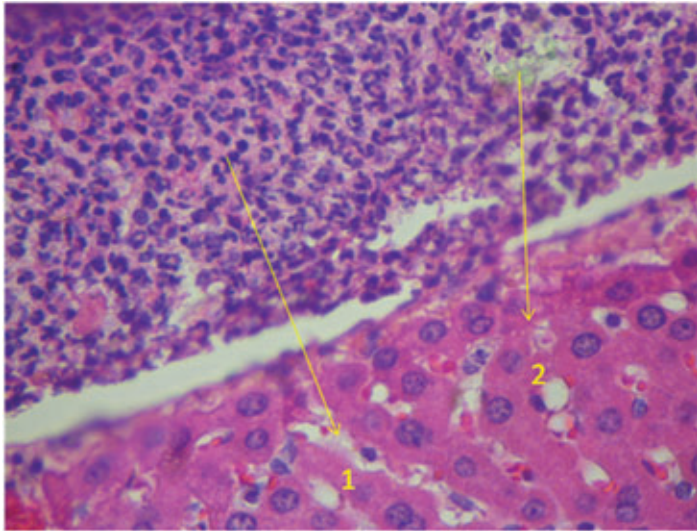
**FIGURE 2** - Lung Group. **A:** bronchopneumonia (Arrow 1) and intra-alveolar hemorrhage (Arrow 2). **B:** exudate in the interior of the respiratory bronchioles (Arrow 1). **C:** macrophage foam cell (Arrow 1) and neutrophils (Arrow 2). **D:** hemosiderophages (Arrow 1).

The fragments extracted from the peritoneum of animals in the control group presented characteristics of diffuse peritonitis (Figure 3A) with intense neutrophilic and plasmacytic infiltrate (Figure 3B and C).



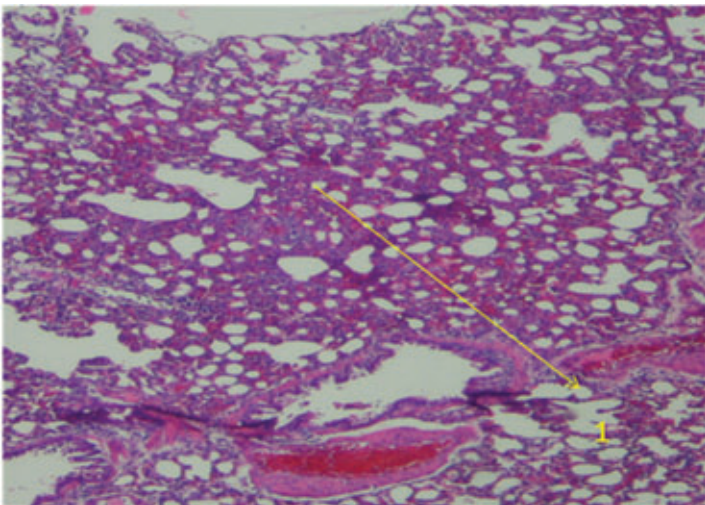
**FIGURE 3** - Peritoneum Group I. **A:** diffuse peritonitis. **B** and **C:** neutrophilic (Arrow 1) and plasmacytic (Arrow 2) infiltrate.

The fragments of rat liver in this group presented with marked acute inflammatory reaction with fibrino-necro-leukocytic exudate in the visceral peritoneum and hepatic capsule surrounding fecal-like material (Figure 4).



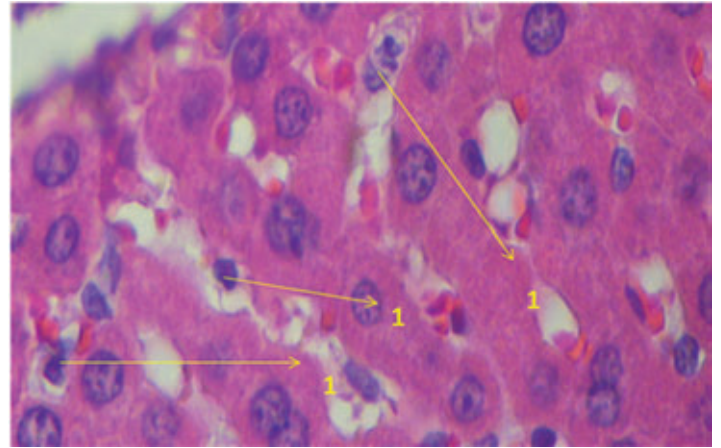
**FIGURE 4** - Liver Group I. Fibrino-necro-leukocytic exudate (Arrow 1) with fecal-like material (Arrow 2).

The animals in the drying group presented with characteristics of pulmonary lesions similar to those of the control group, with diffuse bronchopneumonia and filling of the alveolar spaces with macrophages, neutrophils, and monocytes (Figure 5) in addition to diffuse alveolar hemorrhage and thickening of alveolar septa.

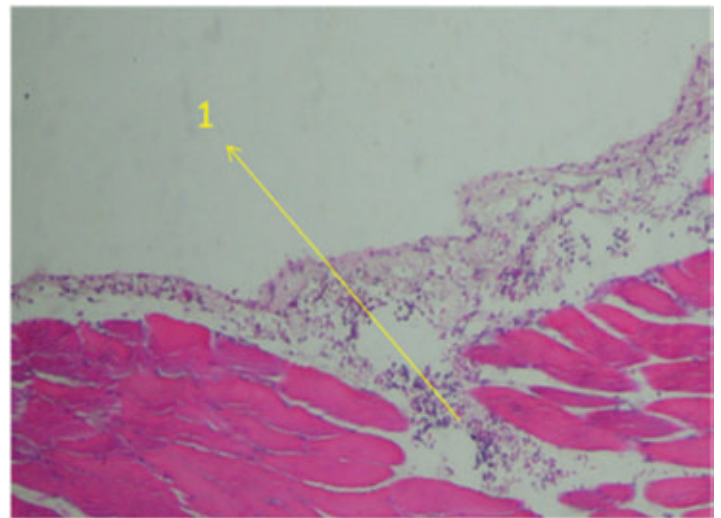


**FIGURE 5** - Lungs Group II. Alveolar spaces with macrophages, neutrophils, and monocytes (Arrow 1).

The fragments extracted from liver and peritoneum of the animals in the drying group also presented characteristics similar to the control group, with intense intra-parenchymal hepatic infiltrate of mononuclear cells (Figure 6) and intense diffuse fibrino-purulent peritonitis (Figure 7).

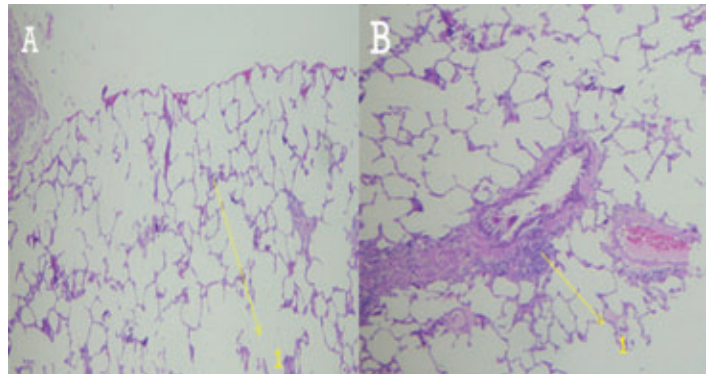


**FIGURE 6** – Liver Group II. Hepatic mononuclear cell Infiltrate (Arrow 1).



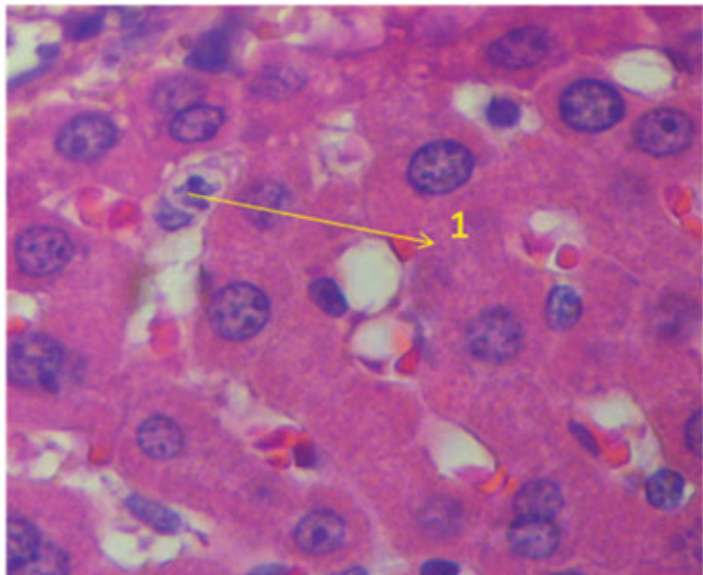
**FIGURE 7** – Peritoneum Group II. Fibrino-purulent diffuse peritonitis (Arrow 1).

The animals in group III presented lungs with preserved architecture, intact alveolar spaces, and discrete and nonspecific alterations (Figure 8A). Pulmonary parenchyma was found with lymphocytic infiltrate in peribronchial nodular disposition (Figure 8B).

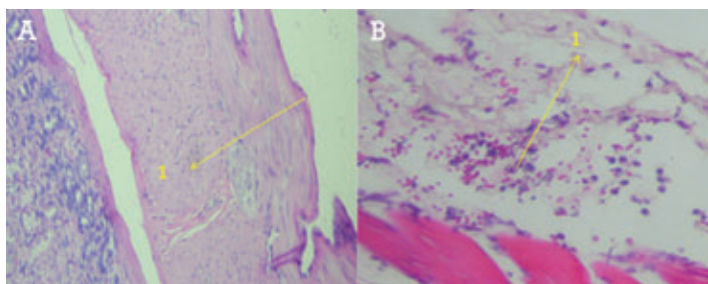


**FIGURE 8** – Lungs Group III. Intact alveolar spaces in in peribronchial nodular disposition (A and B – Arrow 1).

The histological sections of the liver also presented preserved architecture with a discrete mononuclear infiltrate (Figure 9). The peritoneum fragments of the animals in this group presented discrete histological alterations (Figure 10A) with neutrophilic and plasmacytic exudate (Figure 10B).

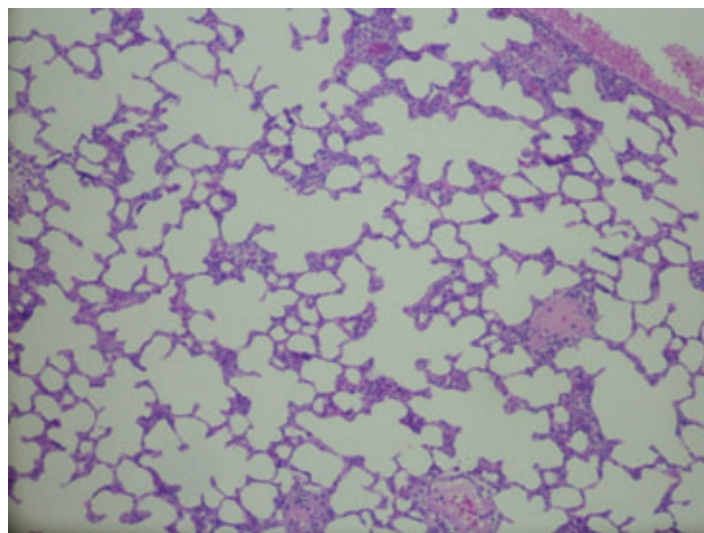


**FIGURE 9** – Liver Group III. Preserved architecture with discrete mononuclear infiltrate (Arrow 1).

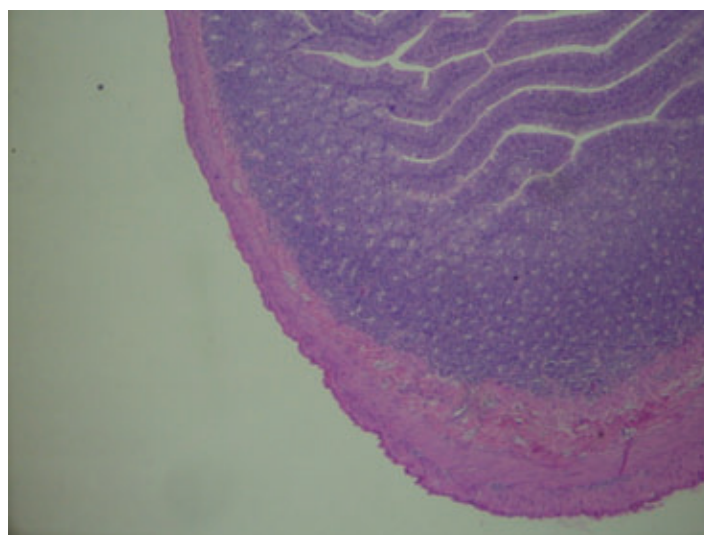


**FIGURE 10** – Peritoneum Group III. Neutrophilic and plasmacytic exudate (A and B - Arrow 1).

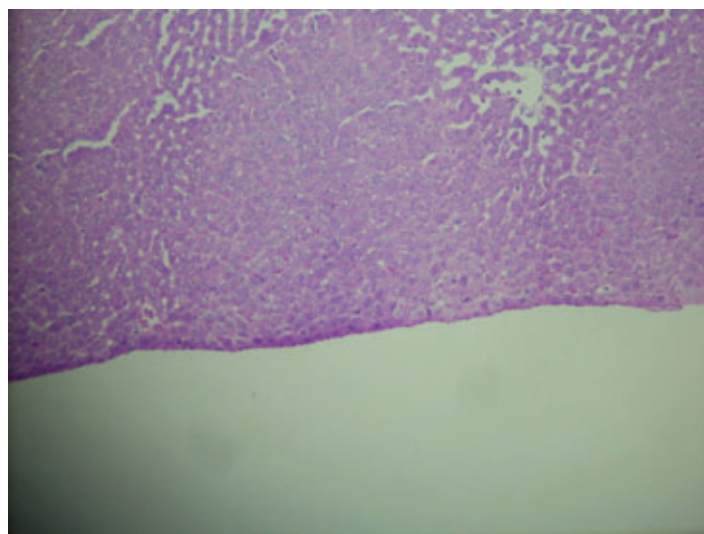
The animals in group IV presented lungs with preserved architecture and intact alveolar spaces that were discrete and nonspecific within the limits of normalcy (Figure 11). The fragments of the visceral peritoneum presented discrete and nonspecific alterations as well (Figure 12). We did not observe any inflammatory response in the several histological sections examined. The hepatic fragments were found to be within the limits of normalcy (Figure 13).



**FIGURE 11** – Lung Group IV. Preserved architecture, intact alveolar spaces.



**FIGURE 12** – Peritoneum Group IV. Discrete and nonspecific alterations.



**FIGURE 13** – Liver Group IV. Findings within the limits of normalcy.

## Discussion

Previous studies demonstrated a reduction in rat fecal peritonitis mortality when the animals were treated with lavage containing lidocaine<sup>8</sup> and bupivacaine<sup>9</sup>. Ropivacaine, despite presenting an anti-inflammatory and antimicrobial effect more discrete than the other anesthetics<sup>10</sup>, also prevented group IV mortality. The histological characteristics of the fragments of the organs evaluated are consistent with the greater survival of group IV animals.

The local anesthetics seem to act in several steps of the inflammatory cascade. Several studies demonstrated a reversible and dose-dependent reduction in the adhesion of leukocytes to the vascular walls<sup>11,12</sup>. Leukocyte migration also seemed to be affected by the anesthetics, most likely due to the action in the cytoskeleton or by attenuation of the release of chemotaxis agents by the leukocytes<sup>13</sup>.

The local anesthetics produce a reversible and dose-dependent inhibition of granulocyte phagocytosis. Systemic intravenous administration of lidocaine at the recommended doses for anti-arrhythmic treatment significantly lowered the phagocytic activity of leukocytes of the synovial fluid of articulations with synovitis<sup>14</sup>. However, experiments with ropivacaine demonstrated discrete or null effects on the phagocytic activity of granulocytes, in contrast to other local anesthetics<sup>10</sup>. The most likely mechanism to explain the inhibition of phagocytic activity is the decrease in expression of the receptor at the surface of leukocytes<sup>15</sup> and inhibition of the activity of actin-myosin filaments<sup>16</sup>.

Despite the controversy surrounding the efficacy of peritoneal lavage in the medical literature<sup>17,18</sup>, our present experiment has shown that peritoneal lavage does lower mortality. The local anesthetics increase survival in several studies on experimental models with mice<sup>19</sup> and dogs<sup>20</sup> even when administered systemically.

Besides the modulatory effects of inflammation, the local anesthetics have proven antimicrobial action. Data in the literature shows that the antimicrobial potency of local anesthetics is essentially correlated with the concentration and, to a lesser degree, the chemical structure, and these anesthetics might be effective against most bacteria when the concentrations are sufficiently high<sup>21</sup>.

The precise mechanism of antibacterial action is not yet clear but can be correlated to the interaction between local anesthetics with the bacterial wall or with macromolecules at the cellular surface of bacteria. Electrostatic interactions between local cationic anesthetics and anionic components in the membrane

could contribute to functional alterations at the cellular membrane level, reducing membrane fluidity<sup>22</sup>.

## Conclusion

The peritoneal lavage with 0.2% ropivacaine, which was used in the treatment of fecally-induced peritonitis with autogenous stool in rats, was shown to decrease the histopathological findings related to the inflammatory response and sepsis.

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