

## Effects of peritoneal lavage with lidocaine on survival of rats with fecal peritonitis<sup>1</sup>

### Efeito da lavagem peritoneal com solução de lidocaína na sobrevivência de ratos com peritonite fecal

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#### ABSTRACT

**Purpose:** To study the effects of peritoneal lavage with a 2% lidocaine solution, on the survival of the rats submitted to peritonitis caused by their own feces. **Methods:** Forty-eight Wistar rats, weighting between 300g and 330g (mean, 311,45 ±9,67g), were submitted to laparotomy 6 hours following induction of fecal peritonitis. Animals were randomly divided into four groups of 12 each as follows: 1- Control, no therapy; 2- Drying of the abdominal cavity; 3- Peritoneal lavage with saline and drying; 4- Peritoneal lavage with a 2% lidocaine solution and drying. Animals that died were submitted to necropsy and the time of their death recorded; survivors were killed on the post-operation 11<sup>th</sup> day and necropsied. **Results:** Death occurred within 52 h in all animals of group 1; within 126 h in 100% of those of group 2; within 50 h in 50% of those of group 3. All animals of group 4 survived. Survival on the 11<sup>th</sup> day was higher in groups 3 and 4 than in groups 1 and 2 (p<0.001), and higher in group 4 than in group 3 (p<0.01). **Conclusion:** Peritoneal lavage with a 2% lidocaine saline solution without adrenaline, prevented the mortality of all animals with fecal peritonitis.

**Key words:** Peritonitis. Anesthesia/methods. Sepsis.

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#### RESUMO

**Objetivo:** Estudar o efeito da lavagem peritoneal com solução de lidocaína a 2% na sobrevivência de ratos com peritonite fecal por fezes autógenas. **Métodos:** Foram utilizados 48 ratos Wistar, pesando entre 300g e 330g (M.A 311,45 ±9,67) submetidos à laparotomia 6 horas após a indução de peritonite, distribuídos aleatoriamente em 4 grupos: 1- (n=12) Controle, nenhum tratamento; 2- (n=12) Enxugamento da cavidade abdominal; 3- (n=12) Lavagem da cavidade abdominal com 3 ml de solução salina 0,9% e enxugamento; 4- (n=12) Lavagem da cavidade abdominal com 30 mg/Kg (± 0,5 mL) de lidocaína 2%, sem adrenalina, e 2,5ml de solução salina 0,9% e enxugamento. Os animais que faleceram foram necropsiados e o horário do óbito anotado. Os sobreviventes foram mortos no 11º dia de pós-operatório e realizou-se a necropsia. **Resultados:** Houve 100% de mortalidade nos animais do grupo 1, em 52 horas; 100% nos animais do grupo 2, em 126 horas e 50% nos animais do grupo 3 em 50 horas. Os animais do grupo 4 sobreviveram. A sobrevivência, no 11º dia de pós-operatório, foi maior nos grupos 3 e 4 em relação aos grupos 1 e 2 (p<0,001) e maior nos grupos 4 que no grupo 3 (p<0,01). **Conclusão:** A lavagem peritoneal com lidocaína a 2% sem adrenalina e diluída em 2,5 ml de solução salina, foi eficaz para evitar o óbito, por 11 dias (eutanásia) em 100% dos animais com peritonite fecal.

**Descritores:** Peritonite. Anestesia/métodos. Sepsis.

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1. Research performed at the Laboratory of the Division of Surgical Principles, Department of Surgery, School of Science, Santa Casa de Misericórdia de Vitória (EMESCAM), Espírito Santo, Brazil.

## Introduction

Despite all advances in the treatment of peritonitis, no decrease in mortality from that disease occurred over the last two decades.<sup>1</sup>

This mortality increases when multiple organ and systemic dysfunctions occur. Although not having a well-elucidated pathogeny, this dysfunction seems to be consequent to a complex inflammatory process. The septic response is associated with the release of anti-inflammatory and inflammatory cytokines<sup>2,3,4</sup>, followed by activation of leukocytes, complement and the coagulation cascade<sup>5</sup>, as well as antibody production and bacteria destruction by polymorphonuclear leukocytes.<sup>6</sup> Mediators like TNF alfa, IL-1 beta, IL-6, IL-8 and e NO, play fundamental roles in sepsis, and the anti-inflammatory mediators concomitantly present, modulate the effects and the release of inflammatory agents.<sup>7</sup>

Local anesthetics have shown to be efficient modulators of the inflammatory cascade in ischemia and heart<sup>8,9</sup>, lung<sup>10,11</sup> and liver reperfusion<sup>12,13</sup>. They are capable of performing an anti-inflammatory action on various cell types, including monocytes, macrophages and neutrophils<sup>14</sup>. Ropivacaine decreased the pulmonary inflammatory response evoked by a lipopolysaccharide in rats<sup>15</sup>. One percent lidocaine and 0.5% bupivacaine were shown to prevent peritonitis caused by 0.1M hydrochloric acid, in comparison with saline<sup>16</sup>. Mice having septic peritonitis induced according to an experimental model<sup>17</sup>, treated with 5% or 10% lidocaine, or 1% or 2% bupivacaine subcutaneously by an infusion pump, decreased mortality and protected rats from hepatic and renal hepatic dysfunction, by attenuating the hiperinflammatory response<sup>18</sup>. Some anesthetics furthermore, presented at the laboratory level, a bactericidal effect against some bacteria<sup>19,20</sup>. Based on these aspects, we questioned whether intraperitoneal application of a local anesthetic dissolved in saline, could improve the survival of animals submitted to peritonitis. The present work was aimed at the verification of the effect on survival from fecal peritonitis, of a 2% lidocaine saline solution without adrenaline (30 mg/Kg), placed in the abdominal cavity of rats.

## Methods

This work was approved by the Ethics Committee of Research of the Faculty of Medicine of the Federal University of Minas Gerais (UFMG), according to protocol 144/06 (COEP-CETEA).

Forty-eight Wistar rats, weighting between 300g and 330g (mean, 311,45 ±9,67g), originating from the Animal House of the Superior School of Science of the Santa Casa de Misericórdia de Vitória-ES (EMESCAM), were anesthetized with KETAMIN-S(+) [S(+)] (ketamine hydrochloride 12,5mg/kg), and submitted to abdominal puncture with "abocath" 16G in the lower left quadrant of the abdomen, and weighted in an electronic balance (Filizola® São Paulo- Brasil) having a 1g sensitivity. Five ml of a suspension of 2g of recently defecated feces, diluted in 17ml of saline, were injected into the abdominal cavity,

after having been filtered through gauze in order to permit free passing through the interior of the needle in the direction of the cavity. Six hours after peritonitis induction, animals were anesthetized with a mixture of xilazine hydrochloride (2,5mg/kg König, S. A. Argentina) and KETAMIN-S(+) [S(+)] (ketamine hydrochloride), 25mg/kg, CRISTALIA®, Itapira, São Paulo, Brasil), and submitted to midline laparotomy of approximately 2 cm length, cavity examination, and collection of 0.5 ml of secretion for bacterioscopy and antibiogram culture. Animals were aleatorily distributed into 4 groups of 12 each as follows: 1-Controls, no therapy; 2- Drying of the abdominal cavity; 3- Peritoneal lavage with 3 ml 0.9% saline and drying; 4- Peritoneal lavage with 30 mg/kg (+/- 0.5 ml) of 2% lidocaine solution without adrenaline, 2.5 ml 0.9% saline and drying. In groups 3 and 4 the saline solution, with or without anesthetic, was left for three min in the cavity, and carefully manipulated between the abdominal viscera to let the anesthetic establish greater contact with the peritoneum and a more efficient action. The peritoneal fluid was then dried with gauze removing most of it, the abdominal wall sutured in two planes with "mononylon" 4-0, simple sewing. On the first plane, the aponeurotic muscles and on the second, the skin were sutured. All animals were subcutaneously rehydrated with a single dose of 10 ml of 0.9% saline every 24 h, for two days. Analgesia was applied by subcutaneous 0.1 mg /Kg nalbuphine hydrochloride every 8 hours for two days.

Animals that had died, were submitted to necropsy, and the time of their death, recorded. Survivors were sacrificed by intraperitoneally-injected 50 mg/Kg of sodium pentobarbital on the 11<sup>th</sup> day post-operation. Secretion at infection sites were collected from the abdominal cavity, for bacteriological examination. Adherences were classified into six grades according to Diogo-Filho *et al*<sup>21</sup>: grade 0- absence; grade 1- a reduced number of adherences of fibrous character, easily disintegrated by manipulation; grade 2- firm adherences resistant to manipulation, located between intestinal loops but not involving the abdominal wall; grade 3- firm adherences, resistant to manipulation, between the abdominal wall and an abdominal organ or structure; grade 4- firm adherences, resistant to manipulation, located between the abdominal wall and more than one organ or structure; grade 5- firm adherences, resistant to manipulation, found between loops and between loops and the abdominal wall, showing an enteric fistula.

Survival frequencies were analyzed by exact Fisher test comparisons between number of survivals per group, and Kaplan Meier survival curves by the Log Rank test. The Kruskal-Wallis analysis of variance was utilized to compare body weights of the 4 animal groups. A value of  $p < 0.05$  was considered significant.

## Results

Laparotomy performed 6 hours following abdominal puncture and injection of a suspension of the recently defecated feces, showed edema, hyperemia between loops and secretion of purulent fluid in the abdominal cavity.

The bacteria isolated from the peritoneal fluid on laparotomy were respectively:

*Proteus mirabilis*, *Klebsiela pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, *Micrococcus*, *Proteus penneri*, *Enterococcus gallinarum*, *Staphylococcus sciuri*, *Bacillus species*, *Staphylococcus epidermidis*, *Aerococcus viridans*. Their sensitivities to antibiotics are shown on Chart 1.

**CHART 1** - Bacteria isolated from the peritoneal secretion of rats submitted to fecal peritonitis and their

Bacteria isolated	Sensitivity
<i>Klebsiela pneumoniae</i>	Amicacin, amp/sulbactam, ampicillin, aztreonam, cefazolin, Cefotetam, ceftiazidone, ceftioxiame, ciprofloxacina, gentamicin, Imipenem, levofloxacina, meropenem, cefepime
<i>Enterococcus faecalis</i>	Synercid, tetracycline, trimeth/sulfa, vancomycin, gatifloxacina, Linezolid
<i>Escherichia coli</i>	Amicacin, amp/sulbactam, ampicillin, aztreonam, cefazolin, Cefotetam, ceftiazidone, ceftioxiame, ciprofloxacina, gentamicin, Gentamicin, imipenem, levofloxacina, meropenem, cefepime.
<i>Micrococcus</i>	Amox/klav, amp/sulbactam, ampicillin, cefazolin, ceftiazidone, Ciprofloxacina, clindamicina, erytromicina, gentamicin, levofloxacina Nitrofurantoin, norfloxacina, oxacilina, penicilina, rifampina
<i>Proteus penneri</i>	Amicacin, amp/sulbactam, ampicillin, aztreonam, cefazolin, cefepime, Cefotetam, ceftiazidone, ceftioxiame, ciprofloxacina, gentamicin, Gentamicin, imipenem, levofloxacina, meropenem.
<i>Enterococcus gallinarum</i>	Amox/klav, amp/sulbactam, ampicillin, cefazolin, ceftiazidone, Ciprofloxacina, clindamicina, erytromicina, gentamicin, Nitrofurantoin, norfloxacina, oxacilina, penicilina, rifampina
<i>Staphylococcus sciuri</i>	Synercid, tetracycline, trimeth/sulfa, vancomycin, gatifloxacina, Linezolid
<i>Bacillus species</i>	Synercid, tetracycline, trimeth/sulfa, vancomycin, gatifloxacina, Linezolid
<i>Staphylococcus epidermidis</i>	Synercid, tetracycline, trimeth/sulfa, vancomycin, gatifloxacina, Linezolid
<i>Aerococcus viridans</i>	Synercid, tetracycline, trimeth/sulfa, vancomycin, gatifloxacina, Linezolid
<i>Morganella morganii</i>	Amicacin, amp/sulbactam, ampicillin, aztreonam, cefazolin, Cefotetam, ceftiazidone, ceftioxiame, ciprofloxacina, gentamicin, Imipenem, levofloxacina, meropenem, cefepime

amp- ampicillin; trimeth- trimethoprim; amox- amoxicilina; klav- clavulina

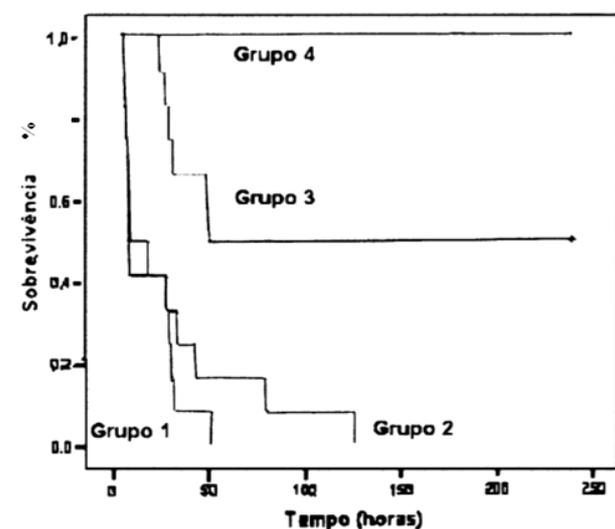
No significant differences between the weights of the 4 groups ( group 1- 313, 3 ± 11,5; group 2- 315,0± 11,7 ; group 3- 307,5± 4,5; group 4- 310,0±8,5 (p=0,36), were apparent.

Rats surviving the operation were dynamic and ingested liquid food. Examination of their abdominal cavity showed 2<sup>nd</sup> and 3<sup>rd</sup> degree adhesions between intestinal loops and the abdominal wall. Rats that died were adynamic, with piloerection, a dark halo around the eyes, tachypneic and anorectic. Examination of their abdominal cavity showed slight purulent secretion and loose adhesions of 0 and first degree between intestinal loops.

Survival was more frequent in group 4 animals in relation to those of group 3 (p<0.01) and of groups 2 and 1 (p<0.001) (Table 1). The survival curves show 100% mortality within 52 hours among animals of group 1, 100% within 126 hours, among animals of group 2, and 50% within 50 hours in animals of group 3. Animals of group 4 survived beyond 11 days. The Log Rank test showed that a significant increase occurred in the survival curve of group 4 relative to group 3 (p<0.01), of group 4 in relation to groups 2 and 1 (p<0.001), of group 3 relative to 1 (p<0.001), and no difference between groups 1 and 2 (Figure 1).

**TABLE 1** - Rats that survived up to the 11<sup>th</sup> day P.O \* following peritonitis induction, submitted to laparotomy followed by respectively : no treatment (group 1-control), drying of the abdominal cavity( group 2); washing of the abdominal cavity with 3 ml of 0.9% saline and drying (group 3); washing of the abdominal cavity with 30 mg/Kg(± 0,5 mL) of 2% lidocaine, without adrenaline, and with 2.5ml of 0.9% saline and drying( group 4)

Groups	No		Death*		
	No	Yes	p1	p2	p3
1- Control	0	12			
2- Drying	0	12	NS		
3- Washing with saline	6	6	0,01	0,01	
4- Washing with Lidocaine	12	0	0,0000	0,0000	0,01
Total	30	30			



**FIGURE 1**- Survival curve of rats submitted to peritonitis and to laparotomy, followed respectively, by: no treatment (group 1-control), drying of the abdominal cavity( group 2); washing of the abdominal cavity with 3 ml of 0.9% saline and drying (group 3); washing of the abdominal cavity with 30 mg/Kg(± 0,5 mL) of 2% lidocaine, without adrenaline, and with 2.5ml of 0.9% saline and drying( group 4). Log Rank test . p<0.05 between group 4 in relation to groups 3, 2 and 1, and group 3 relative to 1

## Discussion

Washing of the abdominal cavity with a 2% lidocaine solution without adrenaline, avoided death of rats submitted to peritonitis caused by their own feces. A similar value had been observed when the peritoneal cavity was washed with an antibiotic to treat peritonitis caused by a lethal dose of *Escherichia coli*.<sup>22</sup> This result did not occur when the abdominal cavity was not treated (control group) or even when it was aspirated (group 2), or aspirated and washed with saline (group 3). It is worth pointing out that in group 3 in which cavity aspiration was followed by peritoneal washing with saline, survival was higher than in the control group. This shows that washing was beneficial in our model. Nevertheless, it is known that peritoneal washing in peritonitis is a controversial issue. Although peritoneal washing is utilized by a large number of surgeons<sup>23</sup>. Peritoneal washing with physiological saline of peritonitic rats, evoked lower mortality compared to plain cleaning of the cavity with compresses<sup>24</sup>. In group 4 rats, when the local anesthetic was added to the saline, a better result was obtained, indicating that the solution with the local anesthetics was more efficient against peritonitis than saline alone. The mechanism by which anesthetics act in these cases is probably, basically, anti-inflammatory. This was well demonstrated by the utilization via the subcutaneous route of a pump for continuous infusions of 5% and 10% lidocaine, or 1% and 2%, bupivacaine respectively, that led to decreased mortality of peritonitic rats<sup>18</sup>. Furthermore, local anesthetics had been shown to be efficient in modulating the inflammatory cascade in heart<sup>8,9</sup>, lung<sup>10,11</sup>, and liver<sup>12,13</sup> ischemia and reperfusion, and were able to exert an anti-inflammatory effect on various cell types including monocytes, macrophages and neutrophils<sup>14</sup>. 1% lidocaine and 0,5% bupivacaine were also able to prevent peritonitis caused by 0.1M hydrochloric acid. Ropivacaine attenuated the inflammatory pulmonary response to lipopolysaccharides in rats<sup>15</sup>. Means to treat sepsis based on the inflammatory component have failed, partly due to their lack of ability to affect the components of the coagulation process. The activation of the cascade of coagulation, has been associated with the progressive failure of multiple organs and systems and the poor prognosis for septic patients. This is probably a consequence of disseminated intravascular coagulation that affects vital blood flow to organs, resulting their failure and death<sup>25</sup>. In this research this fact was not important in the animals treated with the local anesthetic, since all survived.

Besides their anti-inflammatory effect, some anesthetics present bactericidal action against some bacteria at the laboratory level<sup>19,20</sup>. In this experiment, the test of sensitivity of bacteria isolated from the abdominal cavity of the rats was not done because in a pilot study it was not defined whether lidocaine presented bacteriostatic or bactericidal effects. Besides the possible anti-inflammatory effect of the anesthetics, it must be considered that the aspiration of the abdominal cavity in group 4 is also a mechanism of combat of peritonitis since it removes bacteria and toxins.

In surviving animals, the abdominal cavity presented more intense adhesions than in those that died. The function of isolating septic processes and protecting the organism from bacteremia has been attributed to the adhesions. Their inhibition is accompanied by higher mortality consequent to a generalized intra-abdominal septic process<sup>26</sup>.

It is worth noting that animals from the control group and from the controls of groups 2 and 3 that died, presented in the immediate post-operative stage, manifestations of sepsis, including tachypnea, anorexia, adynamic behavior, piloerection and a dark halo around the eyes, as already related<sup>27</sup>. Surviving animals on the other hand, were active and searched for food. It is important to recall that rats that survived until the 10<sup>th</sup> day, did not die from peritonitis, as could be verified in pilot studies in which they were sacrificed after this period for macroscopic examination of the abdominal cavity. This ten day period was utilized as the parameter for the statistical analysis of survival. Considering that there was no statistically significant difference between animal weights in the 4 study groups, that the animals were of the same gender and species, and that the same technique of peritonitis was employed, one can in a certain way set up survival comparisons between the different groups.

The dose of the anesthetic utilized was minimal when compared with the mean lethal dose ( $LD_{50}$ ) of lidocaine, of 111,0 to 133,1 mg/Kg<sup>28</sup>. In the peritoneal washing performed following aspiration, the anesthetic was manually spread within the cavity, to insure that the drug had the greatest possible contact with all viscera, to in this way act more amply. The anesthetic solution was made to remain about 3 min in the cavity in order to have sufficient time to act; for aspiration it was tried to dry the cavity with gentle moving, avoiding to remove all of the solution. This method was satisfactory, since no death occurred in group 4, in which the anesthetic was utilized. Further work for the study of other anesthetics at the same doses in other peritonitis models<sup>7,29</sup>, associated or not with other therapeutic resources, could be developed. The effect of each local anesthetic on bacteria-evoking peritonitis, on the function of organs and on the inflammatory reaction prior to and after the application of these drugs, can be the object of future studies. Two worries leave doubts in the surgeon's mind: the degree of involvement of the mesothelial cells (peritoneum), and the real value of the peritoneal washing during experimental peritonitis.

## Conclusion

Peritoneal washing with a 2% lidocaine solution without adrenaline, diluted in 2.5 ml saline, was effective in avoiding death in 100% of rats with fecal peritonitis.

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### **Acknowledgment**

To the Department for Support of Clinical and Experimental Research of the Institute for Sustainable Development (Instituto Solidário do Espírito Santo), for financial support.

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Conflict of interest: none  
Financial source: In acknowledgment

Received: August 23, 2007  
Review: October 22, 2007  
Accepted: November 20, 2007

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### **How to cite this article**

Brocco MC, Paulo DNS, Baptista JFA, Ferrari TA, Azevedo TCV, Lázaro da Silva A. Effects of peritoneal lavage with lidocaine on survival of rats with fecal peritonitis. *Acta Cir Bras.* [serial on the Internet] 2008 Jan-Feb;23(1). Available from URL: <http://www.scielo.br/acb>

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