

TREATMENT OF ABDOMINAL SEPSIS WITH LEVAMISOLE AND PERITONEAL LAVAGE IN RATS

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ABSTRACT

Introduction: Intra-abdominal sepsis is one of the most difficult situations in surgery and usually presents as a peritonitis. It is a systemic inflammatory response to infection that is often associated with hypoperfusion followed by tissue injury and organ failure. Given that its the sequelae reflect the increasing severity of the systemic response to infection, a correct and early treatment needs to be established. In this sense, the study analyzes the effect of peritoneal lavage and the use of the isomers immunomodulator in the treatment of abdominal sepsis in rats. **Methods:** The animals were randomly assigned to three groups A (control), B (peritoneal lavage), C (levamisole). All groups underwent laparotomy, anesthesia and cecal ligation/puncture. A ten-parameter clinical score was analyzed, which allowed the classification of sepsis degree, peritoneal fluid cultures, and cytokine dosage. **Results:** Group C had better scores on the clinical score and also lower growth of bacterial colonies in cultures. A lower dosage of TNF-alpha, IL-1b, IL-6 and PCR in group C was observed, compared to groups A and B. **Conclusion:** Levamisole positively influenced the treatment in the parameters analyzed compared to the peritoneal lavage group and the control group.

Key words: Abdominal sepsis. Peritoneal lavage. Levamisole. Treatment. Rats.

RESUMO:

Introdução: A sepse intra-abdominal é uma das situações mais difíceis na cirurgia e geralmente se apresenta como peritonite. Trata-se de uma resposta inflamatória sistêmica à infecção que frequentemente associada à hipoperfusão seguida de lesão tecidual e falência orgânica. Tendo em vista que as suas sequelas refletem a gravidade crescente da resposta sistêmica à infecção, é necessário estabelecer um tratamento correto e precoce. Nesse sentido, o estudo analisa o efeito da lavagem peritoneal e o uso do imunomodulador levamisol no tratamento da sepse abdominal em ratos.

Métodos: Os animais foram distribuídos randomicamente em três grupos A (controle), B (lavagem peritoneal), C (levamisol). Todos os grupos foram submetidos à laparotomia, anestesia e ligadura/punção cecal. Foi analisado um escore clínico com dez parâmetros o qual permitiu classificar o grau de sepse, além de culturas do líquido peritoneal e dosagem de citocinas. **Resultados:** O grupo C obteve melhor pontuação no escore clínico e também menor crescimento de colônias bactérias nas culturas do que os grupos A e B. Dosagens significativamente menores das citocinas TNF-alfa, IL-1b, IL-6 e PCR foram observadas nos ratos do grupo C em comparação com os grupos A e B, respectivamente. **Conclusão:** O levamisol influenciou positivamente no tratamento nos parâmetros analisados em comparação com o grupo da lavagem peritoneal e o controle.

Palavras-chave: Sepse abdominal. Lavagem peritoneal. Levamisol. Tratamento. Ratos.

INTRODUCTION

Intra-abdominal sepsis is one of the most challenging situations in surgery and usually presents as peritonitis¹. Gastrointestinal perforation, with leakage of alimentary contents into the peritoneal cavity, is a common surgical emergency and may have life-threatening sequelae¹. Mortality from sepsis by viscera perforation increases with delayed diagnosis and treatment. The 30-day mortality rate after emergency abdominal surgery in Great Britain has ranged from 3.6% in top-performing hospitals to 41.7% in poorer hospitals².

In developing countries, the overall mortality rate has been reported as less than 17%³. This shows that the surgical outcome depends on a complex interaction of many factors and the success obtained with the early initiation of specific therapeutic procedures. Mortality is low when operations are conducted by experienced anesthetists and surgeons, with immediate access to intensive care treatment after surgery. The "surgeon factor", in other words, a decision making about surgical management of the acute abdomen, is a critical determinant of outcome².

Interindividual variation, in the pattern of mediator release and responsiveness of vital organs, may play a significant role in determining the initial physiological response to severe sepsis, which may be a key determinant of outcome. It all depends on the initial severity of the infection, as it occurs in the perforation of the colon, the

speed in the management of treatment attempts, the general health of the patient and the consequent ability to resist the process⁴.

Surgical peritonitis may emanate from perforation, ischemia (mesenteric or strangulation), pancreatitis, and anastomotic fistulas. Considering the pathophysiology, sepsis is an evolutionary process. It is a systemic inflammatory response to infection often associated with hypoperfusion, followed by tissue damage and multiple organs failure. Therefore, its sequelae reflect the increasing severity of the systemic response to infection and not severity of infection¹.

It is difficult to practice and obtain the optimal diagnosis and early surgery for the abdominal sepsis, reducing morbidity and mortality to zero as patients and diseases are variable. However, like infection, inadequate tissue perfusion and persistent inflammatory status are the most important risk factors for the development of multiple organ failure. It seems logical that early therapeutic efforts should be directed to early treatment⁵.

In view of the severity of this disease, it is necessary to establish a correct and early treatment. Early-onset of broad-spectrum antibiotics has been critical during the systemic inflammatory response phase for the prevention of sepsis and septic shock. It has been clearly demonstrated that patient mortality is significantly lower when appropriate antibiotics are prescribed early for the disease⁶. The literature points out that Levamisole, an antihelminthic drug in clinical practice, has an immunomodulatory effect by activating T lymphocytes by Th1 response and inhibiting suppressor T cells⁷. This effect on cellular immunity is important in the pathophysiology of helminth infestations, but it can also have an important effect on bacterial infections that progress to sepsis.

In agreement that abdominal sepsis is a serious and common condition that compromises immune defenses, it is important to study new conducts and medications to help controlling this disease.

The objective of this study is to analyze and compare the effect of peritoneal lavage and the use of levamisole in the treatment of abdominal sepsis induced by ligation and cecal puncture in rats.

METHODS

The protocol was approved by the Ethics Committee on Animal Use (protocol no. 002/17) All experimental procedures were performed based on the guidelines of Law 11,794 (CONCEA).

Experimental design

Wistar rats weighing 278 ± 25 g were randomly assigned to three groups. Each group with six animals. Group A (control), treated with saline, Group B with intraperitoneal lavage and Group C with levamisole 10 mg/kg.

Sepsis induction

All rats were anesthetized with ketamine (70mg/kg) and xylazine (10mg/kg), intraperitoneally. After antisepsis with 70% ethyl alcohol, the animals were submitted to a 4 cm median laparotomy with exteriorization of the cecum, which was ligated with 2-0 cotton thread. Four perforations were made in the cecum using a 25F needle. Gentle pressure was applied to allow a small amount of fecal material to escape into the drilling holes. After repositioning the cecum in the abdominal cavity, the abdomen was sutured in two layers with 4-0 nylon thread.

After 24 hours of sepsis induction, re-laparotomy was performed for culture of peritoneal fluid in Cromoclin US medium, and blood collection for serum measurement of TNF α (pg/mL), IL1- β (pg/mL), IL- 6 (pg/mL) and C-reactive protein (mg/dL).

Clinic score

After 48 hours of post-sepsis observation, all rats had their clinical score assessed⁸ based on the parameters contained in table 1. For each parameter, 1 point was assigned, and in the absence of the analyzed parameter, 0 point. Then, the points were calculated for each mouse. A score of 0 indicated that the animal did not present any clinical alteration, a score between 1 and 3 was considered mild sepsis, between 4 and 7 moderate sepsis and between 8 and 10 severe sepsis.

Table 1 – Clinic score

Clinic score	Individual score
Piloerection	1
Change in breathing rate	1
Fecal chance	1
Lacrimal and or eyelid changes	1
Contraction of the abdomen	1
Lack of strength when grabbing	1
Change in body temperature	1
Alert Response	1
Motor activity impairment	1
Commitment to environmental exploration	1

1-3: mild sepsis; 4-7: moderate sepsis and 8-10: severe sepsis

Treatment

In group B animals, peritoneal lavage was performed after collecting the material for culture, with 0.9% saline solution, heated to 36.5°C. It was repeated three times,

each with 5 ml of solution, which remained in the peritoneal cavity for 1 minute, being removed with sterile dry gauze. In animals of group C levamisole 10 mg / kg was injected intraperitoneally, the first dose being the day before the cecal puncture and two doses on consecutive days after induction of sepsis.

Statistical analysis

Statistical evaluation was performed using the Kruskal-Wallis test followed by Dunet's multiple comparison test. Comparisons were considered significant when $p < 0.05$.

RESULTS

All animals survived the procedures. Those in group A obtained a clinical score 7, being classified as having moderate sepsis; Group B was considered moderate sepsis in the same way, however, they reached a score of 5. And animals in group C, with a score of 3, were considered as low sepsis. Thus, in relation to the clinical results, which evaluated the physical state of the rats, the animals in group C obtained lower results than the other groups.

Table 2 also shows that according to the results of the cultures, the lowest value of *E. Coli* colony formed units occurred in group C (levamisole), with significant differences in relation to groups A and B ($p = 0.001$). In addition to the clinical and microbiological aspects, lower doses of TNF α , IL-1 β , IL-6 and C-reactive protein were observed in animals in group C respectively compared to groups A and B ($p = 0.001$). (Data summarized in table 2)

Table 2 – Results of studied variables

Variable	Grup A (Control)	Grup B (peritoneal lavage)	Grup C (Levamisole)	p-value
Clinic score	7	5	3	0.001
<i>E. coli</i> colonies (UFC/ml)	76	14	4	0.002
TNF- α (pg/mL)	617.6 \pm 17	371.95 \pm 12	187.1 \pm 9	0.001
IL-1b (pg/ mL)	176.4 \pm 10	88.75 \pm 7	46.65 \pm 5	0.001
IL-6 (pg/ mL)	136.9 \pm 8	67.5 \pm 6	57.7 \pm 4	0.001
PCR (mg/dL)	9.65 \pm 0.3	5.05 \pm 0.3	3.85 \pm 0.2	0.001

DISCUSSION

Through the results, the study demonstrated that levamisole resulted on better results in sepsis than did peritoneal lavage. Sepsis is defined as an infection with evidence of systemic inflammation, consisting of two or more of the following: increase

or decrease in temperature or leukocyte count, tachycardia and rapid breathing. Infections of the chest, abdomen, genitourinary system and primary bloodstream cause more than 80% of cases of sepsis⁹. For the installation of the infection, there is a synergism between aerobic microorganisms, with *Escherichia coli* that reduce the oxygen content and facilitate the growth of anaerobes, such as *Bacteroides fragilis*, and the presence of contaminants such as feces, bile or urine¹⁰. Providing cultures and prescribing properly antimicrobials are crucial therapeutical measures¹¹.

In addition to the use of antibiotics, some measures are important for the control of this complex and lethal disease, the abdominal sepsis. In surgery, peritoneal lavage is frequently used for cases of peritonitis¹² and has become a common measure in surgery with the use of various types of fluids. The most commonly used is the 0.9% saline. In the present study, a comparison was made between the peritoneal lavage and the use of levamisole. The study showed that Group C (levamisole) had better results, with statistical significance, in relation to the number of *E. coli* colonies, having a significant impact on the treatment.

In the course of any infection, the production of mediators is necessary and physiological; however, it is believed that excessive or prolonged activation of these cellular and humoral mediating pathways contributes to the development of multiple organ failure in patients with severe sepsis¹³. The early proinflammatory state in severe sepsis results in a later and prolonged damage of the immune system. Blood of Spleen collected from ICU patients who had active sepsis when they died were depleted of CD4+ and CD8+ T cells, and splenocytes showed marked reductions in stimulated cytokine production¹⁴. The loss of CD4+ and T cells appears to be mainly the result of apoptosis¹⁵ which prevents patients from developing an adequate response to infection¹⁴. Thus, a drug with an immunomodulatory effect could help the control of sepsis. It directly interferes with the pathophysiology of sepsis, which is the pro-inflammatory status. This effect was observed with levamisole in this study.

Levamisole is a synthetic *phenylimidazolthiazole* widely used as anthelmintic and works as an immunomodulator preventing the action of suppressor T cells. Levamisole has been shown to increase late hypersensitivity reactions, mitogenic response and *in vitro* lymphocyte proliferation. It is more effective in cellular immunity, as it stimulates T lymphocytes more than B lymphocytes. This drug induces stronger Th1 immune responses through the activation of dendritic cells or inducing the expression of the Toll-like receptor⁷. In studies conducted in animals infected with enterotoxic *Escherichia coli*, levamisole has been shown to influence the proliferation of T cells in the ileal villi, stimulating the differentiation and proliferation of lymphocytes in the lamina propria and in the Peyer plates¹⁶.

Levamisole can also affect the differentiation and proliferation of M cells, increasing their number in the intestinal villi. Historically, almost all attention has been

focused on M cells and their interactions with the immune cells in Peyer's plaques, the main defense inducing sites in the intestine^{16,17}.

Levamisole has been shown to stimulate the proliferation of M cells in the ileal villi and may be relevant in the process of cellular immunity¹⁷. In accordance with the already known effect of levamisole, the results in table 2 of the present study also allow us to believe that group C obtained better results also in the pro-inflammatory response. Thus, this effect of cellular immunomodulation proved to be important for the control of infection, considering that animals treated with levamisole obtained superior clinical results.

CONCLUSION

In conclusion, treatment with levamisole resulted in better clinical and laboratory results in rats with abdominal sepsis when compared with peritoneal lavage and control. These results may have implications in the clinical setting, as it points to a possible use of this drug as a new adjuvant for the control of abdominal sepsis.

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