

EFFECT OF PNEUMOPERITONEUM IN MODEL OF ABDOMINAL SEPSIS

EFEITO DO PNEUMOPERITÔNIO EM MODELO DE SEPSE ABDOMINAL

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ABSTRACT

Purpose: This study aims to examine the effects of CO₂ pneumoperitoneum on the level of inflammation biomarkers in a model of abdominal sepsis in rats. **Methods:** Twelve Wistar rats were randomly assigned to two groups of 6 rats each. Under anesthesia, abdominal sepsis was induced by cecal ligation and puncture (CLP). Rats were observed for 24 hours. Group 1: sepsis; group 2: sepsis + pneumoperitoneum with CO₂ for 30 min. Six hours after pneumoperitoneum deflation, blood samples were collected by cardiac puncture to measure (TNF α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), C-reactive protein (CRP) and procalcitonin (PCT). Statistical analysis using Student t test (p<0.05).

Results: All animals survived. The rats with sepsis + pneumoperitoneum had levels of TNF α , IL-1 β and IL-6 (542.4 \pm 21 pg/mL, 312.1 \pm 12 pg/mL and 115.4 \pm 11 pg/mL, respectively), significantly lower than in the sepsis group (p = 0.01). Serum cytokine levels in the sepsis group were TNF α = 733.2 \pm 24 p /mL; IL-1 β = 432.1 \pm 15 pg/mL and IL-6 = 232.3 \pm 16 pg/mL. Serum levels of PCT and CRP were significantly lower in the sepsis + pneumoperitoneum group than in the sepsis group. (p=0.01). **Conclusion:** In conclusion, pneumoperitoneum CO₂ attenuates the biomarkers of the acute phase inflammatory response associated with perioperative sepsis by PLC.

Key words: Sepsis. Pneumoperitoneum. Cytokines. Procalcitonin. C-reactive protein. Inflammation.

RESUMO:

Objetivo: Este estudo tem como objetivo examinar os efeitos do pneumoperitônio de CO₂ no nível de biomarcadores de inflamação em um modelo de sepse abdominal em ratos. **Métodos:** Doze ratos Wistar foram distribuídos aleatoriamente em dois grupos de 6 ratos cada. Sob anestesia, em todos os animais foi induzida sepse abdominal por ligadura e punção do ceco (LPC). Os ratos foram observados por 24 horas. Grupo 1: LPC sepse; grupo 2: sepse + pneumoperitônio com CO₂ por 30 min. Seis horas após a desinsuflação do pneumoperitônio, amostras de sangue foram coletadas por punção cardíaca para dosagem de (TNF α), interleucina-1 β (IL-1 β), interleucina-6 (IL-6), proteína C reativa (PCR) e procalcitonina (PCT). Análise estatística pelo teste de Student ($p < 0,05$). **Resultados:** Todos os animais sobreviveram. Os ratos com sepse + pneumoperitônio com CO₂ tiveram níveis de TNF α , IL-1 β e IL-6 (542,4 \pm 21 pg/mL; 312,1 \pm 12 pg/mL e 115,4 \pm 11 pg/mL, respectivamente), significativamente mais baixos que no grupo sepse ($p = 0,01$). As dosagens séricas das citocinas no grupo sepse foram TNF α =733,2 \pm 24 pg/mL; IL-1 β =432,1 \pm 15 pg/mL e IL-6=232,3 \pm 16 pg/mL. Os níveis séricos de PCT e PCR foram significativamente mais baixos no grupo sepse + pneumoperitônio que no grupo sepse. ($p = 0,01$). **Conclusão:** Em conclusão, o pneumoperitônio de CO₂ atenua os biomarcadores da resposta inflamatória de fase aguda associada à sepse peri-operatória por CLP.

Descritores: Sepse. Pneumoperitônio. Citocinas. Procalcitonina. Proteína C reativa. Inflamação.

INTRODUCTION

Sepsis is defined as a life-threatening organ dysfunction that occurs due to a dysregulated host response to infection¹. Sepsis is one of the most common causes of ICU hospitalization, and its frequency continues to increase². Several studies have shown that mortality related to sepsis has been decreasing annually, but in low and middle-income countries there is still a high mortality rate that can be up to 80%. Patients at risk of sepsis should be identified prior to the onset of organ dysfunction and they require rapid diagnosis and onset of treatment¹. The immune system harbors humoral, cellular, and neural mechanisms that attenuate the potentially harmful effects of the proinflammatory response³. Phagocytes can switch to an antiinflammatory phenotype that promotes tissue repair, and regulatory T cells and myeloid-derived suppressor cells further reduce inflammation. In addition, neural mechanisms can inhibit inflammation. In animal models of sepsis⁴, disruption of this neural-based system by vagotomy increases susceptibility to endotoxin shock, whereas stimulation of the efferent vagus nerve or $\alpha 7$ cholinergic receptors attenuates systemic inflammation⁵. Some patients frequently have ongoing infectious foci, despite antimicrobial therapy⁶. Multiple studies have documented reduced responsiveness of blood leukocytes to pathogens in patients with sepsis³, findings that were recently corroborated by postmortem studies revealing

strong functional impairments of splenocytes obtained from patients who had died of sepsis in the ICU. Besides the spleen, the lungs also showed evidence of immunosuppression^{5,6}.

In the activated innate immune response to sepsis, pro-inflammatory and anti-inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and monocyte chemoattractant protein 1 (MCP-1) are released, followed a rise in the levels of acute phase proteins such as procalcitonin and C-reactive protein^{7,8}.

Several experimental evidences suggest that CO₂ pneumoperitoneum reduces the severity of sepsis and prolongs survival, compared with laparotomy in animal models of abdominal sepsis⁹⁻¹¹. However, little is known about the CO₂ pneumoperitoneum impact on early acute phase response in sepsis and respective biomarkers. Some experimental models have been successful in the study of abdominal sepsis¹²⁻¹⁴. Considering these knowledges, we performed this study, with the objective of examine the effects of CO₂ pneumoperitoneum on the level of inflammation markers and survival in a rat model of abdominal sepsis.

METHODS

Wistar rats (*Rattus norvegicus*) 3 to 4 months old were used, from the Vivarium of the Health Sciences Center of the Federal University of Rio Grande do Norte. The protocol was submitted for appreciation and approved by the HUOL Animal Use Ethics Committee (CEUA / HUOL) in accordance with protocol No. 03/2019. The Wistar rats were kept in individual polypropylene cages with 12 h light-dark cycles. They went through a 7-day acclimatization period at the Experimental Surgery Center-UFRN, with ad libitum access to water and food for rats (Presence[®]). The care in the use of animals followed the rules of the Brazilian Legislation for the scientific use of animals (Law n^o 11.794 / 2008).

Animals and groups

The rats were anesthetized with intraperitoneal injection of ketamine 70 mg/kg and xylazine 7 mg/kg. After epilation and antisepsis of the abdomen with 70% alcohol, ligation and puncture of the cecum was performed in all animals.

Experimental design

Twelve rats were randomly assigned to two groups of 6 rats each.

Group 1: sepsis group. In all animals, abdominal sepsis was done by cecal ligation and puncture. After anesthesia and aseptic procedures with 70% ethanol, a 4 cm laparotomy was performed. The cecum was exposed, ligated with sterile 2-0 cotton and four punctures were made with a 25 gauge needle. A small amount of fecal material was

extruded through the punctures, and the cecum was pushed into the abdomen. The abdominal wall was sutured with nylon 4-0 (Ethicon) in two layers. All rats received volemic reposition of 5 mL of sterile saline subcutaneously.

Group 2: sepsis + pneumoperitoneum (30 min)

Sixteen hours after CPL, a CO₂ pneumoperitoneum was created using a Veress needle connected to a pressurized container. CO₂ was insufflated into the peritoneal cavity using an automatic insufflator (Edlo, RS, Brazil) until the intra-abdominal pressure stabilized at 10 mmHg. The pneumoperitoneum was maintained for 30 min, after which the needle was removed and the abdomen was deflated. All surgical procedures were performed using aseptic technique.

Biochemical analysis

Six hours after pneumoperitoneum deflation, the surviving rats were anesthetized with ketamine (70 mg/kg) + xylazine (7 mg/kg) i.p. Blood samples were collected by cardiac puncture and centrifuged at 3500 rpm for 10 min and the serum was stored at -20°C until analysis. Then the rats were euthanized with overdose of thiopental (100 mg/kg i.p.).

The following dosages were processed: tumor necrosis factor-alpha (TNF α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), using the ELISA technique with kits from PeproTech, Rocky Hill, NJ, USA, according to the manufacturer's recommended protocols. Fluorescence was measured using the Bio-Tec Instruments EL 808 microplate reader, using the KC4-V3.0 software. The detection sensitivity was 20 pg/ml for cytokines. The serum concentration of procalcitonin (PCT) was determined in duplicate using an immunoluminometric assay (Brahms, PCT-LIA, Berlin, Germany). The lowest concentration of PCT detectable by this technique was 0.08 ng/mL and the reference value established by the method was <0.5 ng/mL. C-reactive protein (CRP) was measured by immunoturbidimetry, using the Orion Diagnostica equipment (OY, Finland), and the reference value established by the method was 0 to 3 mg/L.

Statistical analysis

All values were expressed as mean \pm standard deviation. The analyzes were performed using the Student's t-test and the p-value <0.05 was considered statistically significant. Statistical determinations were performed using the BioEstat 5.0 software.

RESULTS

All animals survived the experiments until the end of the observation period.

Rats with sepsis associated with CO₂ pneumoperitoneum had levels of TNF α , IL-1 β and IL-6 (542.4 \pm 21pg / mL; 312.1 \pm 12 pg / mL and 115.4 \pm 11 pg / mL respectively) lower than the animals submitted only to CPL; the difference was statistically significant

($p = 0.01$). The serum dosages of cytokines in the sepsis group were, (TNF $\alpha = 733.2 \pm 24$ pg/mL; IL-1 $\beta = 432.1 \pm 15$ pg/mL and IL-6 = 232.3 ± 16 pg/mL. Data summarized in table 1.

Table 1 - Cytokine dosage values of rats with abdominal sepsis associated or not with CO₂ pneumoperitoneum.

Cytokines	Sepsis + pneumoperitoneum	Sepsis	P-value
TNF α (pg/ml)	532.4 \pm 21	733.2 \pm 24	0.01
IL-1 β (pg/mL)	312.1 \pm 12	432.1 \pm 15	0.01
IL-6 (pg/mL)	115.4 \pm 11	232.3 \pm 16	0.01

Cytokine dosages differ significantly between groups ($p = 0.01$, Student's t-test).

Serum levels of procalcitonin (PCT) were significantly lower in septic animals with pneumoperitoneum, compared with animals with only sepsis, as can be seen in Table 2. The levels of C-reactive protein (CRP) followed the same interpretation PCT, that is, rats with sepsis and pneumoperitoneum had significantly lower serum CRP levels than animals submitted to PLC alone ($p = 0.01$). Data summarized in table 2.

Table 2 - Values of procalcitonin and C-reactive protein in rats with abdominal sepsis associated or not with CO₂ pneumoperitoneum.

Acute phase proteins	Sepsis + pneumoperitoneum	Sepsis	P-value
PCT (ng/ml)	4.2 \pm 1.3	7.8 \pm 1.4	0.01
CRP (mg/L)	13.1 \pm 1.1	19.7 \pm 1.5	0.01

CRP, C-reactive protein; PCT, procalcitonin. CRP and PCT dosages differ significantly between groups (Student's t-test).

DISCUSSION

The major pathophysiological mechanism accounting for the development and progression of sepsis is the dysregulation of dynamic balance between inflammatory mediators and anti-inflammatory factors¹⁵. Infection, without early treatment, usually leads to severe persistent hypotension, and affects the physiological functions of multiple organs, contributing to the septic shock and multiple organ dysfunction syndrome, with high mortality^{16,17}.

With regard to cytokines, TNF- α is released first, and can activate signal to induce the secretion of pro-inflammatory IL-1 β and IL-6 cytokines, and inhibit the anti-inflammatory factors, acting to trigger the release of inflammatory cascade¹⁸.

PCT is a sepsis marker prohormone, which represents the precursor to calcitonin. Serves as an indicator with a high value in monitoring sepsis in clinical practice¹⁹. Sepsis

is always associated with an acute increase in PCT. Generally, a few hours after infection, PCT can be detected in serum, and peak level is usually soon released. It has been confirmed that PCT can serve as an effective indicator for diagnosis of infection in critical patients and to monitor the management of sepsis. An increasing in PCT can be detected in severe sepsis and septic shock²⁰⁻²². PCT is not released in the presence of non-infectious inflammation or localized infection²². In fact, in the present study, we observed that PCT had very high serum levels in rats with sepsis and that the association of pneumoperitoneum with sepsis resulted in a significant decrease in PCT levels.

Significant prevalence of high concentrations of CRP in critically ill patients has been described²³. In this study, the association of pneumoperitoneum in animals with abdominal sepsis by CLP contributed to the decrease in serum CRP levels, compared to the group without pneumoperitoneum, meaning that the pneumoperitoneum with CO₂ exerted a reducing effect on the expression of sepsis biomarkers such as CRP and proinflammatory cytokines. In the present study, the finding of a lower CRP serum levels in the abdominal sepsis + CO₂ pneumoperitoneum group than in the sepsis group showed that CO₂ pneumoperitoneum contributed to decreasing the acute phase inflammatory response against sepsis. Corroborating our results, Are et al demonstrated similar findings²⁴.

It has been stated that CO₂ pneumoperitoneum may cause elevated hypercapnia and toxic shock syndrome in cases with abdominal sepsis²⁵. However, it has also been demonstrated that pneumoperitoneum has no impact on the systemic dissemination of abdominal sepsis and that laparoscopic surgery preserves peritoneal defense mechanisms²⁶. In conclusion, CO₂ pneumoperitoneum attenuates the biomarkers of acute phase inflammatory response associated with perioperative CLP sepsis.

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