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EVALUATION OF PULMONARY CHANGES CAUSED BY INDUCED SEPSIS AND THE USE OF PROPRANOLOL IN ENDOTHELIAL DYSFUNCTION IN WISTAR RATS

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Abstract: Objective: To evaluate the potential effect of propranolol as a possible lung protector on endothelial dysfunction in cases of induced sepsis in Wistar rats. Methods: The animals were divided into 3 groups: Group 1, intraperitoneal propranolol administration 30 minutes before sepsis induction; Group 2, administration of intraperitoneal propranolol 30 minutes after sepsis induction; Group 3, SHAM (control), septic animals without propranolol administration. For evaluation, hemorrhage, edema, atelectasis and pneumonia scores were used as tissue damage criteria. Results: Propranolol was found to protect against pneumonia and atelectasis, but increased bleeding while edema remained mild in all groups. Final considerations: The results showed that the treated groups showed clinical improvement in different aspects during the sepsis period, with a survival of treated animals when compared to untreated ones.

Keywords: LPC; Beta-blocker; Pneumonia; Atelectasis; Bleeding.

INTRODUCTION

Sepsis is a clinical syndrome characterized by a dynamic and complex state comprised by the interaction between a pathogen and the dysfunctional and exacerbated organic response of the host. Even with the scientific clarification currently available, it is estimated that 2.8 million deaths are attributed to sepsis (MANETA et al., 2023).

The lung is often one of the first organs to be affected. In sepsis, excessive production of catecholamines occurs, which can lead to endothelial dysfunction and multiple organ failure. It is known that endothelial dysfunction affects the lung parenchyma, increasing the susceptibility of developing edema. The extravasation of inflammatory exudate into the alveoli, in addition to causing difficulty in breathing, leads to fibrosis when

not properly treated. (PARK et al., 2019; VIGNESHWARAN et al., 2019).

According to Özyılmaz et al. (2019), beta-blockers have the potential to reduce metabolic changes induced by catecholamines and exacerbated inflammatory responses associated with sepsis, indicating positive therapeutic effects. In the study, they used propranolol as a substance to protect against endothelial dysfunction prior to sepsis. Thus, the objective of the present study was to evaluate the effect of propranolol, a non-selective β-blocker, before and after the onset of sepsis, in an attempt to attenuate endothelial dysfunction and pulmonary condition in septic rats.

REVIEW OF LITERATURE

SEPSIS

Sepsis is a systemic inflammatory response to an infection. The main bacteria involved are: Staphylococcus aureus (Gram-positive), Pseudomonas spp. and Escherichia coli (Gram-negative). In addition to bacteria, some fungi may be involved, with Candida albicans being the main agent found (GRONDMAN et al., 2020).

According to Chakraborty (2019), sepsis is marked by an exaggerated activation of anti-inflammatory mediators and release of inflammation cells. The unregulated release of inflammatory cytokines can lead to the systemic inflammatory response syndrome due to the imbalance between the pro-inflammatory and anti-inflammatory cascade and, consequently, triggering endothelial dysfunction, a condition in which the vascular endothelium is damaged and loses its physiological properties. normal.

To induce sepsis, the model of ligation and puncture of the cecum is widely used, which produces a picture of peritonitis by creating a focus of infection in the cavity, leading to organ dysfunction, hypothermia and metabolic changes (ÖZYILMAZ et al., 2019; KORNEEV, 2019). Peritonitis can occur locally, in an abdominal region, or diffusely. In addition, it can be classified as primary, secondary or tertiary. The primary occurs by sowing, arising from other organs affected by local disease, the secondary occurs when a contaminant irritates the peritoneal lining by direct contact, the tertiary is classified as a recurrence of the secondary, has a more aggressive characteristic, leads to severe sequelae and often to death (CLEMENTS, et al., 2021).

LUNG IN SEPSIS

Endothelial dysfunction in the lung, caused by sepsis, increases capillary permeability resulting in accumulation of protein-rich fluid in the interstitial spaces (edema). The alveolar epithelial barrier is also affected, the interstitial fluid invades the alveolus, surpassing the drainage capacity of the lymphatic system. These changes result in incompatibility of perfusion and ventilation, arterial hypoxemia and possible development of acute respiratory distress syndrome (DELFRATE et al., 2021).

A reactive secondary mechanism involves hypoxic pulmonary vasoconstriction (HPV), with the aim of protecting compromised areas, aiming to increase systemic perfusion. First, there is an instantaneous increase in pulmonary artery pressure, but if hypoxia persists, there is an increase in HPV (PRITESH et al., 2020). These effects of sepsis on tissue perfusion and microvasculature in general can cause multiple organ failure (DELFRATE et al., 2021).

β-ADRENERGIC RECEPTORS

There are three subtypes of β-adrenergic receptors: β1, β2 and β3. β1 receptors are predominantly found in the heart; β2 are found in smooth muscle, in vessels and bronchi; and

β3 are found in adipose tissue (ERASMUS et al., 2019; PINHEIRO et al., 2021).

β1 receptors are responsible for increasing cardiac output, heart rate, ejection fraction and the release of renin by juxtaglomerular cells; in addition, they are also associated with lipolysis, as well as β3 receptors. β2 receptors are predominant in smooth muscle and cause visceral relaxation. Among some known functions are: smooth muscle relaxation, for example, in the bronchi and histamine release by mast cells (ERASMUS et al., 2019; ALMONFREY et al., 2020; NEVES et al., 2023).

β-ADRENERGIC BLOCKERS AND PROPRANOLOL

Adrenergic beta-blockers form a therapeutic class that has beta-adrenergic receptor blockade as a common mechanism of action, but with different pharmacological profiles. Differences are related to the selectivity of beta-adrenergic receptors, liposolubility and vasodilator actions of some drugs in the class (PINHEIRO et al., 2021). Some have vasodilator effects due to different actions, such as alpha-1 adrenergic receptor antagonism or increased nitric oxide release (ALMONFREY et al., 2020). They also affect blood pressure, primarily through their effects on cardiac output, and perform bronchiolar constriction by inhibiting sympathetic bronchodilator activity.

More than 40 years ago, Berk et al. (1975) demonstrated that beta-blockers can reduce mortality from sepsis and experimental and clinical shock. This hypothesis has recently been revised with new data demonstrating metabolism-related benefits for therapy with this class of drugs in experimental clinical treatment settings. Recent studies have demonstrated that beta-blockers may have new therapeutic potential against various hypermetabolic states, attenuating catecholamine-induced metabolic changes and excessive inflammatory responses (BRUNNING et al., 2021).

Propranolol and related β-blockers are similar in structure with a secondary amine substituted for isopropyl on the carbon side chain, shown in Figure 1. This is important for effective interaction with the β receptor (SRINIVASAN, 2019). Propranolol is a non-cardioselective β-blocker. It is reported to have membrane stabilizing properties but lacks intrinsic sympathomimetic activity. It causes minimal depression of heart rate, myocardial contractile force and cardiac output during normal conditions (PINHEIRO et. al., 2021). However, during the action of the sympathetic nervous system, relative bradycardia, decreased myocardial contractility and reduced cardiac output are produced.

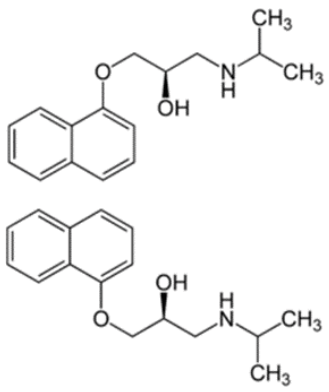

Figure 1: Structural formula of propranolol

Propranolol has immunomodulatory capabilities resulting from pro-inflammatory mobilization related to persistent hypercatecholaminemia. The action of propranolol acts both to increase the levels of serum cell surface immunoglobulins and to reduce the neuroendocrine response to stress, expression of procytokines, granulocyte colony-stimulating factors and neutrophil elastase, which results in the prevention of exacerbated release of cytokines present in sepsis (KARAALI et al., 2023; DOOBAY et al., 2021).

MATERIAL AND METHODS

The project was approved by the UENF Animal Use Ethics Committee (CEUA) under protocol number 161.

ANIMALS

Wistar male rats weighing between 300 and 400g were used. The animals were weighed for the administration of propranolol, diluted in dimethylsulfoxide (DMSO), according to the group to which they would belong. The animals received a standard diet and water ad libitum, and remained under normal living conditions (ambient temperature 20 ± 2 ºC, 45-55% humidity and 12h dark/light cycle).

ANESTHETIC PROTOCOL

The chosen anesthetic protocol was the association of ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, at a dose of 100 mg/kg, and xylazine, an alpha-2-adrenergic agonist, at a dose of 5 mg/kg, intraperitoneally (IP).

SURGICAL TECHNIQUE

After anesthesia, we performed a wide trichotomy of the abdominal region and local antisepsis. The animals were positioned in dorsal decubitus where we made a median incision for laparotomy. Subsequently, there was exposure of the cecum with subsequent use of the model of ligation and puncture of the cecum (CLP) to induce sepsis.

In group 1 (G1) treatment with 10 mg/kg of propranolol was administered intraperitoneally and after 30 minutes the CLP technique was performed.

In group 2 (G2) the CLP technique was performed and after 30 minutes treatment with 10 mg/kg of propranolol was administered intraperitoneally.

In group 3 (G3), the sham group (control), only the CLP technique was performed, without the administration of propranolol treatment.

After the animals presented sepsis, they were euthanized and lung tissue was collected, in which they were placed in 10% formalin for fixation. The material was sent to the Pathological Anatomy Laboratory of ``Universidade Estadual do Norte Fluminense`` (UENF) for histopathological examination (CCTA-LMPA).

STATISTICAL ANALYSIS

For each continuous variable, normality was verified using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between groups were made using Student's t test and analysis of variance (ANOVA) for normally distributed data, and the Whitney U test and Kruskal-Wallis test were used for normally undistributed data. Bonferroni correction was applied for multiple comparisons. Data were expressed as mean ± standard deviation (SD) and as median (min-max); p value <0.05 was considered statistically significant. Statistical analysis was performed using the GraphPad Prism 5.0 program.

RESULTS AND DISCUSSION

The present study evaluated the following lung injury scores among the different groups: hemorrhage, pneumonia, atelectasis and edema (figure 2).

As it can be seen in Figure 3, all animals in group 1 and 2 presented hemorrhage; probably due to the vasodilator action of propranolol, since in group 3 such alteration was not observed (ALMONFREY et al., 2020). When we compared this variable, we observed that in group 1 the hemorrhage scores are slightly lower than in group 2.

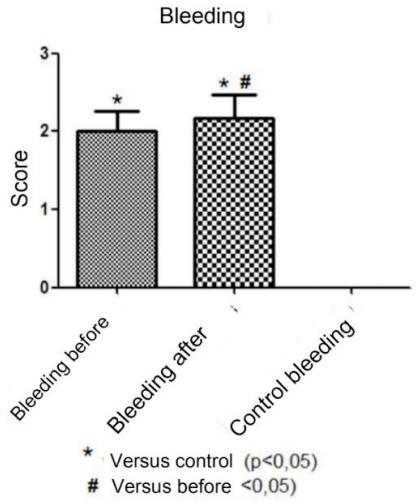

Figure 3: Evaluation scores of the hemorrhage variable in male Wistar rats after the CLP sepsis model. Alterations are observed in animals treated with propranolol and in the control group.

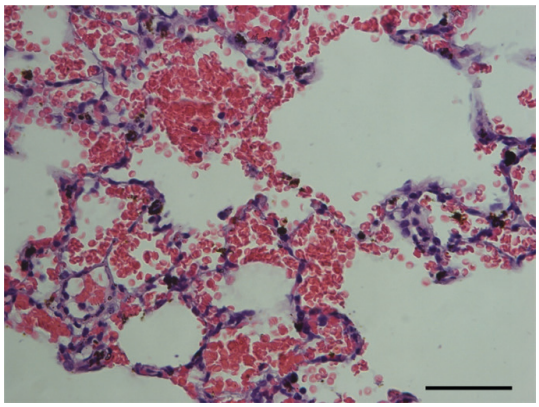

Figure 4: Lung parenchyma of a male Wistar rat submitted to the CLP sepsis model, group 1. Moderate multifocal hemorrhage is observed, indicated by the arrow in the image (4x magnification).

Regarding the pneumonia variable (figure 5), all animals presented some degree of this lesion, and in the treated groups a reduction of the condition was observed. This may have occurred due to the protective action of propranolol on inflammation (ÖZYILMAZ et al., 2019). Other works such as Karaali et al. (2023) cite the action of propranolol in modulating the immune response mediated by the β-adrenergic receptor, reducing the activation of mononuclear cells and therefore of a wide variety of pro-inflammatory cytokines.

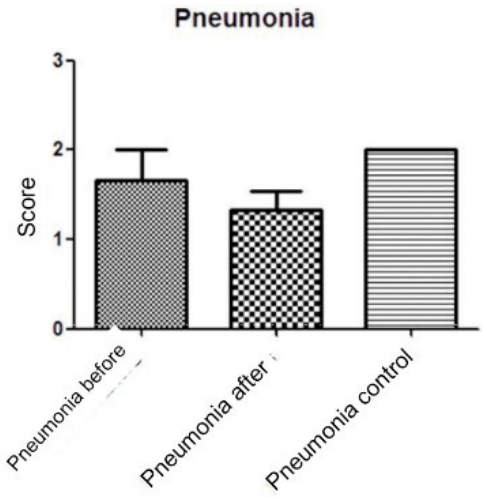

Figure 5: Assessment scores for the pneumonia variable in male Wistar rats after the CLP sepsis model. Alterations are observed in animals treated with propranolol and in the control group.

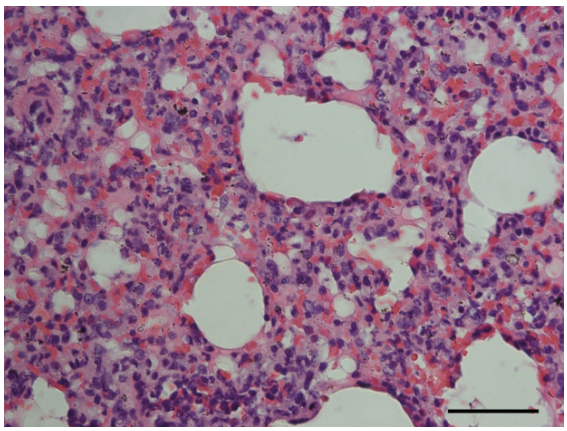

Figure 6: Lung parenchyma of a male Wistar rat submitted to the CLP sepsis model, group 2. Acute diffuse pneumonia with the presence of neutrophils is observed (1x magnification).

Propranolol has pulmonary bronchoconstrictor action due to its action on β receptors (SRINIVASAN, 2019). This is confirmed when we look at group 3 (control), which has a lower score for atelectasis (figure 7). Therefore, we understand that propranolol may have increased the occurrence of

atelectasis due to bronchoconstriction, due to its action on β2 receptors.

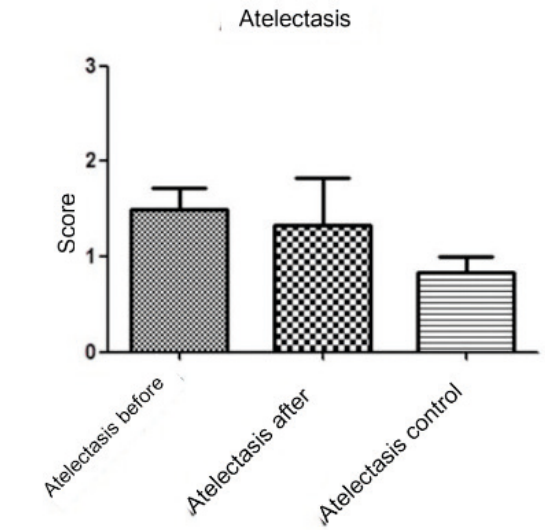

Figure 7: Assessment scores for the atelectasis variable in male Wistar rats after the CLP sepsis model. Alterations are observed in animals treated with propranolol and in the control group.

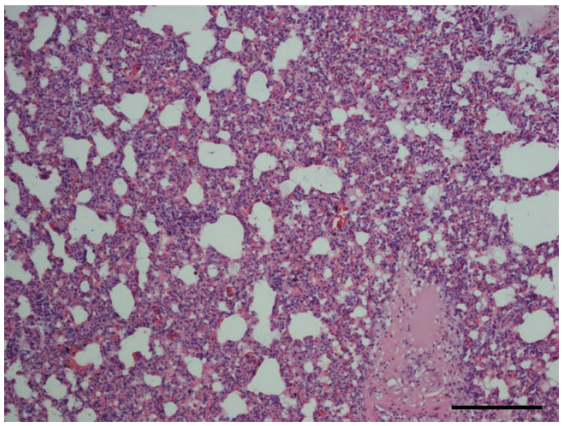

Figure 8: Lung parenchyma of a male Wistar rat submitted to the CLP sepsis model, group 2. Diffuse atelectasis is observed (1x magnification).

When we evaluated the edema variable, no significant alterations were observed between groups, remaining mild in all groups (Figure 9). An improvement in the vascular issue was expected in relation to this factor, since beta-blockers normally improve endothelial dysfunction (ÖZYILMAZ et al., 2019).

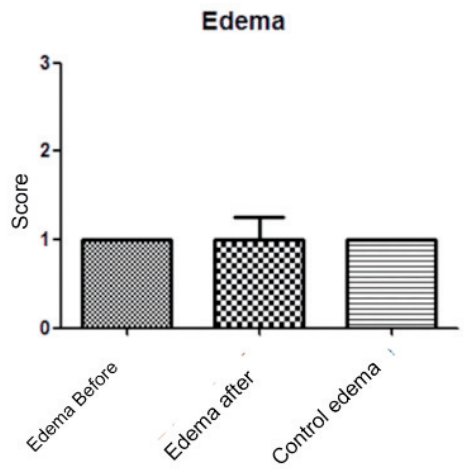

Figure 9: Edema variable evaluation scores in male Wistar rats after the CLP sepsis model. Alterations are observed in animals treated with propranolol and in the control group.

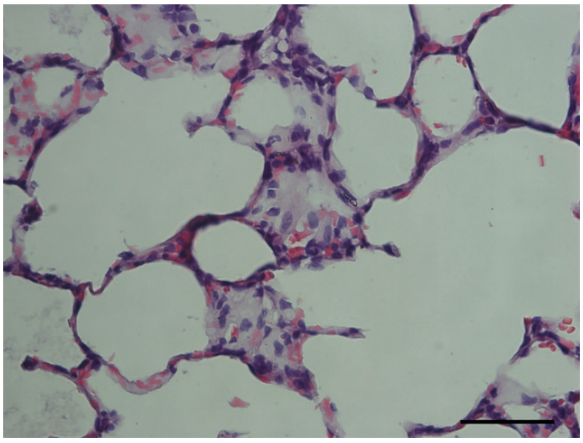

Figure 10: Lung parenchyma of a male Wistar rat submitted to the CLP sepsis model, group 1. Mild multifocal edema is observed, indicated on the image by arrows (4x magnification).

Another important factor analyzed among animals in group 1 was a positive correlation between pneumonia and hemorrhage; that is, the greater the hemorrhage, the greater the pneumonia (R=0.775). In group 2 animals, a weak positive correlation between edema and pneumonia was observed (R=0.612). In group 2, compared to group 1, atelectasis and pneumonia were reduced.

In the correlation tests, a negative

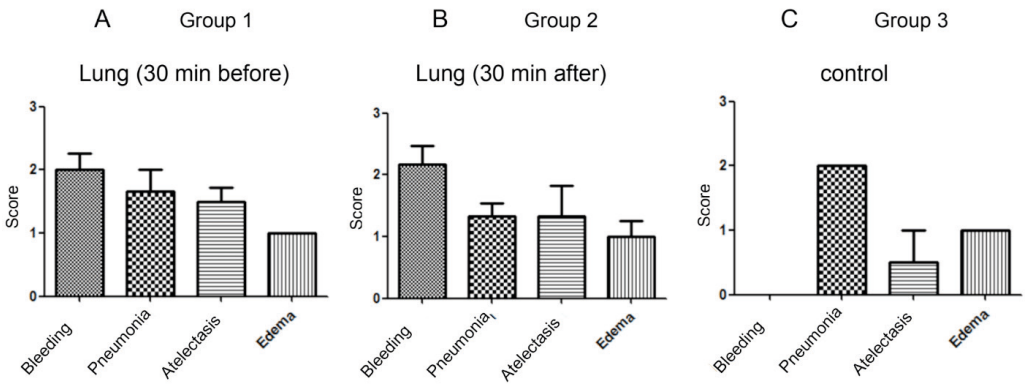

Figure 2: Assessment scores of lung injury classification variables in male Wistar rats after the CLP sepsis model. In A, changes are observed in animals from group 1, administered before CLP; in B, the alterations in the animals of group 2, of administration after the CLP, are observed; in C, animals from group 3 are observed, performing CLP without treatment.

correlation was observed between atelectasis and edema in the animals of group 2; that is, the greater the edema, the lower the atelectasis (R=0.783). A weak correlation was also observed between animals with atelectasis in group 3 compared to treated animals in group 2 (R=0.605).

CONCLUSIONS

The present study showed that the systemic administration of the non-selective β-adrenergic blocker propranolol exerts a protective effect on systemic inflammation, coagulation and survival, as a survival of treated animals was observed in relation to untreated ones. Our data demonstrated that, when administered, it can present a clinical reduction in pneumonia, despite statistically non-significant results. Another important factor is that in the treated group it was observed that pulmonary atelectasis increased when compared to the control group. Edema remained mild in all groups. More experiments and clinical studies are needed to lead to a complete understanding of the mechanism by which propranolol acts in sepsis, allowing not only a new treatment option, but also an improvement in patient survival.

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