Evaluation of the nociceptive threshold and inflammatory markers in rats after induction of pulmonary emphysema by elastase in the phasic, inflammatory and neuropathic pain models

Avaliação do limiar nociceptivo e marcadores inflamatórios em ratos após indução de enfisema pulmonar por elastase nos modelos de dor fásica, inflamatória e neuropática

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ABSTRACT

BACKGROUND AND OBJECTIVES: Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction, although it compromises the lungs, it also produces significant systemic consequences. The objective of this study was to analyze the relationship between pro-inflammatory cytokines and nociceptive threshold in rats with porcine pancreatic elastase-induced COPD.

METHODS: 144 animals were randomly distributed into 3 different models: nociceptive phasic pain at tail-flick test (TF), inflammatory pain in the Freund's complete adjuvant model (CFA) and neuropathic pain in the sciatic nerve constriction model (CCI). 21 days after tracheal instillation of elastase the COPD was established, and the nociceptive threshold was evaluated at different times.

RESULTS: The animals with COPD in TF had a shorter latency time and higher levels of IL-1β, IL-6 and TNF-alpha cytokines. In the CFA and CCI model, the animals with COPD showed an increase in the mechanical hyperalgesia and the levels of IL-1β, IL-6 and TNF-alpha were greater in plasma up to 24 hours.

CONCLUSION: Animals with COPD have higher levels of pro-inflammatory cytokines and reduced nociceptive thresholds, suggesting a relationship between COPD and increased nociception.

Keywords: Chronic obstructive pulmonary disease, Pain, Pulmonary disease.

RESUMO

JUSTIFICATIVA E OBJETIVOS: A doença pulmonar obstrutiva crônica (DPOC) caracteriza-se pela obstrução do fluxo aéreo e, embora comprometa os pulmões, produz importantes consequências sistêmicas. O objetivo deste estudo foi analisar a relação entre citocinas pró-inflamatórias e limiar nociceptivo em ratos com DPOC induzida por elastase pancreática suína.

MÉTODOS: Cento e quarenta e quatro animais foram distribuídos aleatoriamente em três modelos diferentes: dor fásica nociceptiva no teste de retirada de cauda (TF), dor inflamatória no modelo de adjuvante completo de Freund (CFA) e dor neuropática no modelo de constrição do nervo ciático (CCI). Vinte e um dias após a instilação traqueal de elastase a DPOC foi estabelecida e o limiar nociceptivo foi avaliado em diferentes períodos.

RESULTADOS: Os animais com DPOC apresentaram menor tempo de latência de retirada e maiores níveis das citocinas IL-1β, IL-6 e TNF-alfa no TF. No modelo CFA e CCI, os animais com DPOC apresentaram aumento da hiperalgesia mecânica e os níveis de IL-1β, IL-6 e TNF-alfa foram maiores no plasma até 24 horas.

CONCLUSÃO: Animais com DPOC apresentam níveis mais elevados de citocinas pró-inflamatórias e limiares nociceptivos reduzidos, sugerindo uma relação entre DPOC e aumento da nocicepção**.**

Descritores: Doença pulmonar, Doença pulmonar obstrutiva crônica, Dor.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the main causes of morbidity and mortality worldwide and smoking is the main precursor of the disease, responsible for up to 50% of

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HIGHLIGHTS

- This is a study about a disease that is on the rise in Brazil and in the world. It is estimated that in Brazil about 30 thousand deaths/year occur due to chronic obstructive pulmonary disease (COPD), being the fifth leading cause of death.
- Animals with COPD induced by tracheal elastase instillation have elevated levels of inflammatory markers.
- Animals with COPD show altered responses to noxious stimuli, suggesting influence of inflammatory markers on nociceptive perception.

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air obstruction in individuals aged over 70 years[1,3]. It is estimated that in Brazil about 30,000 deaths/year occur due to COPD, and it's the fifth main cause of death. Moreover, data suggests that there are approximately three million people with COPD in Brazil generating about 280,000 hospitalizations per year[4].

The pulmonary respiratory disease known as COPD, has as its main characteristic chronic reduced airflow, which is not fully reversible. Consequently, is progressive and associated with an abnormal inflammatory response in the lungs, which is mainly caused by the inhalation of toxic gases, specially by cigarette smoke[5,6].

The chronic inflammatory process can produce changes in the bronchioles, bronchi, and/or lung parenchyma, which can result in chronic bronchitis, obstructive bronchiolitis, and pulmonary emphysema. However, such changes is different for each individual, being related to the symptoms presented[5,7]. Furthermore, the consequences of COPD experienced by the patient include, weight loss, exercise intolerance, dyspnea, increased energy expenditure, reduced quality of life and financial expenses, which can lead to death[7,8].

Although COPD compromises the lungs, it also produces systemic complications[5]. Thus, COPD can be considered a disease of the respiratory system with systemic manifestations[9]. Moreover, it is a disease with extrapulmonary effects that contribute to their severity[5].

Studies demonstrate that patients with COPD have higher levels of inflammatory markers in the blood, as C-reactive protein (CRP), fibrinogen, tumor necrosis factor alpha (TNF-α) and inflammatory cytokines such as interleukin (IL-6 and IL1-β)[9-11]. Inflammatory markers would be responsible for reducing the pain threshold in patients with COPD, however such evidence is not yet described.

Pain is defined as ''an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage". This pain usually originates in the peripheral nervous system and is processed and interpreted by the central nervous system[12].

The immune system and pain mutually influence each other, moreover the cells involved in the inflammatory response can trigger effects of chronic hyperexcitability and changes in the phenotypic expression of nociceptors, leading to an abnormal processing of harmful signals and exacerbation of the painful sensation[13,14]. Thus, high levels of inflammatory markers involved in the pathogenesis of COPD can influence the systemic manifestations of the disease, however it is not known the impact of these markers in the nociceptors sensibilization, that might increase the painful sensation.

The cells involved in the COPD are T lymphocytes (predominantly CD8+ lymphocytes), macrophages and neutrophils. The activation of these cells triggers the release of inflammatory mediators, mainly leukotriene LTB4, IL-6, IL-8 and TNF-α. Patients with stable COPD have elevated lung concentrations of IL1-β and TNF-α[15-17].

The objective of this study was to evaluate the interaction between the release of pro-inflammatory cytokines in different pain models in animals with porcine pancreatic elastase-induced COPD.

METHODS

The present study followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) recommendations for animal experimentation[18].

Study design

Flow design from the study performed with COPD rats is shown in figure 1.

Sample size

List of animals distributed in their respective groups is shown in table 1.

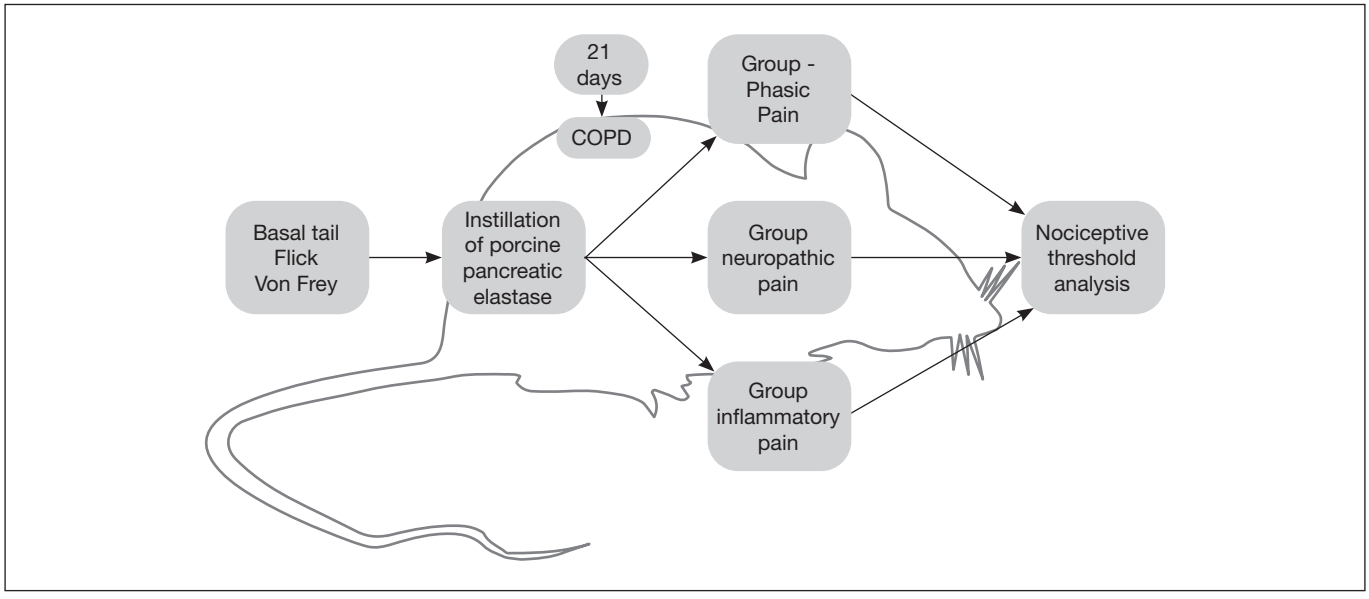

Figure 1. Design of the study carried out in Wistar rats and distribution of groups.

Table 1. Animals distributed

Groups		Animals
Tail-flick	Tail-Flick saline	16
	Tail-Flick saline cytokine	8
	Tail-Flick elastase	16
	Tail-Flick elastase cytokine	8
Neuropathic pain	CCI-Sham	16
	CCI-Sham cytokine	8
	CCI	16
	CCI cytokine	8
Inflammatory pain	CFA-Sham	16
	CFA-Sham cytokine	8
	CFA	16
	CFA cytokine	8

Inclusion and exclusion criteria

This study included male Wistar rats, being 7 to 8 weeks old, weighing between 250 to 300 g, in normal physiological conditions with no physical abnormalities. Furthermore, animals were excluded if they did not present body weight and age within the description, as well as if they presented any physiological alteration.

Randomization

In this study, animals were randomly distributed into the following 3 groups: phasic pain, inflammatory pain and neuropathic pain.

Blinding/masking

The researchers were blinded at the time of the laboratory analysis. Consequently, analyzes of inflammatory cytokines was performed by an examiner external to the study, and the he who performed the analysis was not aware of which group the animal belonged to.

Outcome measure

The following parameters were evaluated, tail flick latency by TF (three consecutive measurements with a 5-minute interval between them, and the measurements were averaged), mechanical nociceptive threshold was evaluated by digital Von Frey test (through a polypropylene tip, applied perpendicularly, to the plantar surface of the hind paw, for a period of approximately 4 seconds or until the animal demonstrates nociceptive behavior, characterized by withdrawal of the paw, followed by licking and/or "flinching", were 3 measurements were collected and the average between them was made).

Statistical analyses

This research used Analysis of Variance (ANOVA two-way), with comparisons established through Bonferroni's post-hoc test and used for data evaluation, which was performed through the Statistical Package for the Social Sciences (SPSS) software (IBM, Chicago, USA) version 15.0. The significance level adopted was $p<0.05$.

Experimental animals

A total of 144 male Wistar rats were obtained from the Central Vivarium of the Federal University of Alfenas (*Universidade Federal de Alfenas* - UNIFAL-MG). Rats were kept under normal conditions in acrylic cages, with temperature between 18-21º C, 55-60% relative humidity, and a 12-hour light/dark cycle. With standard feed and water available until the day of the experiment. The ethical standards established for experimentation with chordate animals, recommended by the IASP (International Association for the Study of Pain) were followed in all experiments[19]. The ethical aspects of the use of animals in the laboratory were prepared and approved by UNIFAL-MG Animal Experimentation Ethics Committee (nº19/2016).

Experimental procedures

Induction of emphysema by porcine pancreatic elastase

Some experimental models were used to simulate COPD: exposure to cigarette smoke, intratracheal instillation of proteolytic enzymes, and strains of genetically modified animals[20]. In the present study, was used the porcine pancreatic elastase model, which in about 21 days after the application of the enzyme, it is already possible to observe lung damage followed by morphological changes, remodeling of the lung parenchyma and impairment of lung function, which made it suitable for to study the alterations provoked in the fibers of the pulmonary parenchyma of the individual with COPD[21].

The animals were anesthetized with ketamine (34 mg/kg, intraperitoneal, i.p.) and xylazine (12 mg/kg, i.p.), an anterior cervical incision was made to expose the trachea, and sterile saline (0.9% NaCl, 5 µL; salt group) or porcine pancreatic elastase (PPE; 0.6 U, Sigma, St. Louis, MO, USA) diluted in 5 µL saline solution (elastase-induced COPD group)[21] were instilled using a sterile Scalp 27 needle. The cervical incision was closed with a suture (4–0 Monocryl; Shalon Medical, Inc).

Nociceptive phasic pain model

Modified in the year 1982 by a study used as reference[22], the degree of antinociception was measured using the TF[21]. On the analgesiometer equipment, with 2 cm from the final portion of the tail, the animal was gently immobilized, thus the nickel-chromium filament progressively heated the animal's tail (approximately 9°C/second) from room temperature (23°C ± 1°C C) until it reached a harmful temperature (~53ºC) in approximately 3 seconds and heating was stopped at 6 seconds to avoid tissue damage. All animals had their baseline threshold evaluated for the TF using three consecutive measurements at 5-minute intervals. After the baseline measurement, the animals underwent tracheal surgery with elastase/saline and after 21 days was submitted to TF at T0, T1, T3, and T24 hours.

Inflammatory pain model

To induce the persistent inflammatory response, the animals received an intraplantar (i.pl) injection of 100 µL of complete Freud's adjuvant (CFA) on the plantar surface of the right hind paw. Mechanical hyperalgesia was assessed using the Von Frey digital technique[23]. Conversely the control group received an i.pl injection of saline (100 µL). Four hours after CFA or saline injection, the mechanical nociceptive threshold was assessed at T0, T1, T3, and T24 hours.

Neuropathic pain model

In the present study the sciatic nerve constriction (CCI) model used was for the evaluation of neuropathic pain[24]. Animals de-

velop behaviors that resemble neuropathy in humans, such as protection of the injured limb (animal tries to hide the paw) suggesting spontaneous pain. Before surgery the animals were anesthetized with ketamine (34 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.). To expose the sciatic nerve, an incision was made in the region below the gluteus where the musculature and skin were retracted. With four ligations with silk thread separated by 2 mm, the nerve was constricted, and the skin was sutured.

At the end of the surgery, the animals were kept warm to prevent hypothermia and then returned to the vivarium. For the animals in the CCI-Sham group, they only had the sciatic nerve exposed and the skin was sutured, not suffering ligation. Three days after the procedure, the animals were tested and considered hyperalgesic when the response threshold to the application of mechanical stimuli corresponded to at least 50% of the baseline response threshold.

Mechanical nociceptive test

The mechanical nociceptive threshold of the animals was evaluated using digital Von Frey (Insight Equipments, Ribeirão Preto, São Paulo, Brazil). Then, the animals were placed in acrylic boxes with the front face positioned on a wire mesh providing access to the hind paw, after approximately one hour for the animals to adapt to the place. Next, the test was performed by the electronic Von Frey apparatus, through a polypropylene tip, applied perpendicularly, to the plantar surface of the left hind paw and at T0, T1, T3, and T24 hours[25]. The force used was sufficient to generate a positive paw withdrawal response[26,27].

Pro-inflammatory cytokines evaluation

Blood collection

Blood was collected by the researcher through cardiac puncture, using the vacuum technique in a sterile environment, where it was stocked and stored for later analysis. After being anesthetized with ketamine (34 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.), the animal was placed on a flat surface, an incision was made in the anterior region of the thorax and a needle was inserted into the left ventricle for blood collection. The tubes were centrifuged at 1500 rpm for 15 minutes after collection. Subsequently, the plasma was removed in a laminar flow hood, using previously sterilized Pasteur pipettes. Subsequently, the samples were placed in sterile Eppendorfs and stored in a freezer at -80°C for analysis. Samples were always collected by the researcher and at the same time, at the beginning of the experiment, after 21 days of application of pancreatic elastase and at T0, T1, T3, T24 hours, which are described in the topic experimental procedures[28].

Plasma levels of inflammatory mediators

ELISA (Enzyme-Linked Immunosorbent Assay) is an enzyme immunoassay that allows the detection of specific antibodies. Moreover, this test is used in the diagnosis of several diseases that induce the production of immunoglobulins. In the ELISA method, the antigen is attached to a surface (generally made of polystyrene) by means of an antibody. Once the antigen bound to the immobilized antibody, this complex can be recognized by another antibody, this time linked to an enzyme that can produce an easily detectable compound. For the analysis of plasma levels of TNF-α, IL-1β and IL-6, 4 milliliters of blood were collected[28].

Analyzes of plasma concentrations of inflammatory mediators were performed using the sandwich ELISA method, using the Quantikine kit (HS, R&D Systems, Minneapolis, USA) to analyze TNF-α IL-1β and IL-6 levels, according to the manufacturer's instructions[15,28].

Experimental groups

Animals were divided into three groups: nociceptive phasic pain group, inflammatory pain group, and neuropathic pain group. In the nociceptive phasic pain group, 16 rats had induced COPD, and other 16 animals (control group) had saline instillation in their trachea. 21 days after the Tail Flick test was applied at T0, T1, T3, and T24 hours, and 16 animals had blood drawn for analysis of the inflammatory markers of the group. In the inflammatory pain group, 16 rats with induced COPD were submitted to CFA, and 16 were submitted to the application of saline, and 16 animals had blood withdrawn for analysis of inflammatory markers. In the neuropathic pain group, 16 COPD rats underwent CCI surgery, and 16 COPD rats underwent sham surgery (CCI-SHAM). In both groups, the von Frey test was performed at their respective T0, T1, T3, and T24 hours and 16 animals had blood drawn for analysis of inflammatory markers.

RESULTS

The evaluated animals belonged to the TF group (32 animals), CFA group (32 animals), CCI group (32 animals) and 48 animals for cytokine dosage and analysis. For the animals evaluated by the TF method, it was possible to observe a statistically significant difference at time 0 ($p<0.05$), which corresponds to the time of the COPD induction surgery through the instillation of porcine pancreatic elastase, but with an interval of 21 days after surgery so that the manifestation of the disease occurred. Such a difference was also observed at T1, T3, and T24 hours.

In figure 2, is possible to observe that the animals with induced COPD, when evaluated by the TF, showed an earlier removal of the tail when compared to the animals in the control group. Moreover, at time 0, the animals with COPD had a latency period of around 2.8 seconds while in the control group the withdrawal was approximately 3.5 seconds. The latency period reduced slightly at T1, T3, T24 hours for the animals in COPD group, showing values close to 2.7 seconds. Moreover, it is possible to observe that COPD rats have different responses to phasic pain when compared to the control group, demonstrating that such rats have reduced nociceptive threshold.

For analysis of cytokines in the TF group, it's possible to observe in figure 3 levels of inflammatory markers close to baseline, since the induction of emphysema had not yet been performed. Nevertheless, after COPD induction, significant differences ($p<0.05$) were found for IL-1β, where at T0 the values of these markers had an evident increase, their plasmatic levels more than doubled in relation to the group of animals without COPD; there was a

slight decline over time, but the statistical difference remained. For IL-6 and TNF-α, this slight reduction in plasma levels did not occur, but there was an increase in the concentration of these markers over time until T24 hours, with statistically significant differences (p<0.05).

When analyzing figures 2 (TF) and 3 (Cytokines), it is possible to observe that in both figures, in T0, there were significant differences (p<0.05), indicating that at the moment of tail withdrawal, the time of latency of animals with COPD was lower and overlaps with higher levels of IL-1β, IL-6 and TNF-α; the same occurs at all times.

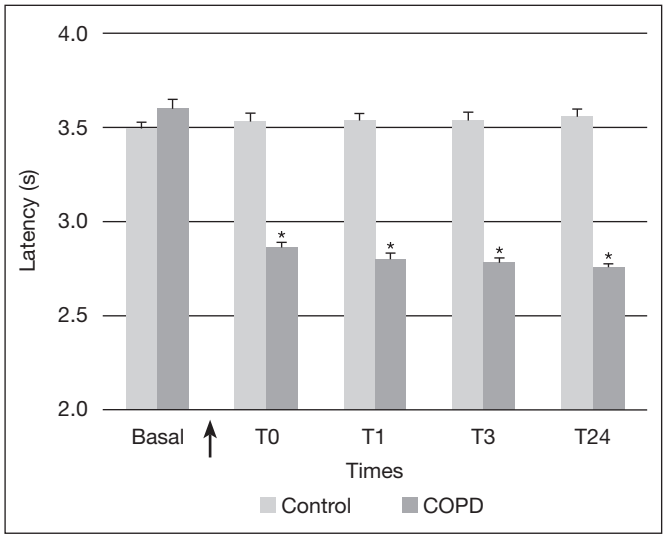

Figure 2. Pain assessment in the phasic pain group, using TF at baseline (before COPD induction), at T0, T1, T3, and later at T24 hours, performed 21 days after induction surgery.

*Significance (p<0.05) in relation to the saline group. The bars were different in relation to the treatment ($F_{1,58}$= 4.21, p<0.01), time ($F_{4,126}$=0.12, p<0.05), and had time x treatment interaction ($F_{8,126}$= 8.83, p<0.05).

In figure 4, it was possible to observe that for baseline values in the CFA groups, at time 0, both the animals with COPD and saline solution induced in the paw presented similar pressure threshold values, indicating that for the mechanical threshold, the presence of COPD did not influence the withdrawal of the paw. Conversely, after the induction of acute hyperalgesia by CFA, animals with COPD showed a lower pressure threshold than saline animals, suggesting that the systemic inflammatory

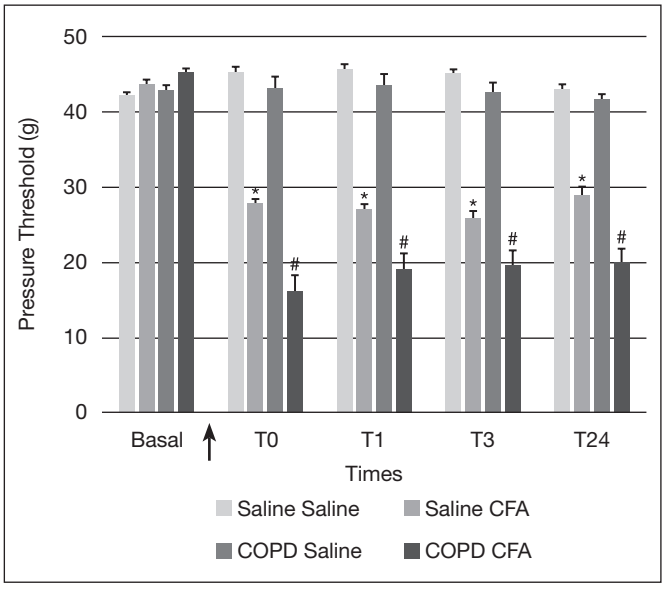

Figure 4. Evaluation of pain in the inflammatory pain group by von Frey test at the following times: baseline (before COPD induction), T0, T1, T3, and later T24 hours, performed 21 days after induction surgery.

*Significance (p<0.05) in relation to the saline group. # Different from all groups (p<0,05). In figure 4, the bars were different in relation to the treatment ($F_{3,20}$= 65.43, p<0.01), time ($F_{4,56}$=19.67, p<0.05) and had time x treatment interaction ($F_{12,56}$= 44.53, p<0.05).

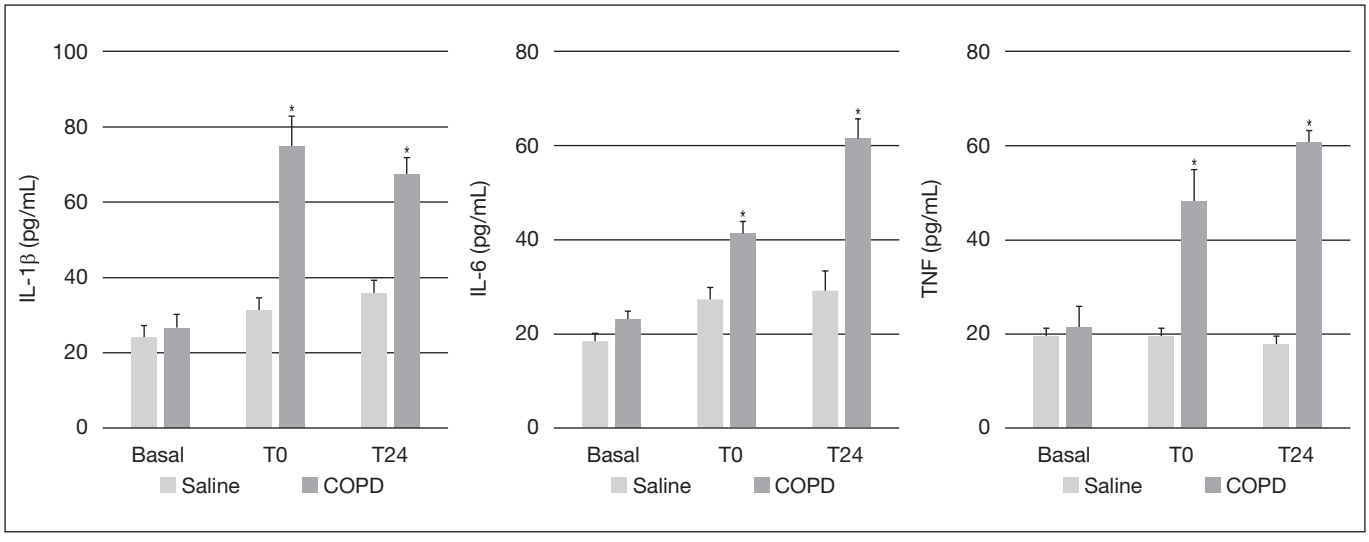

Figure 3. Evaluation of the inflammatory markers on animals belonging to TF group, performing the withdrawal of blood at the following times: baseline (before COPD induction), T0, T1, T3, and later T24 hours, performed 21 days after induction surgery.

*Significance (p<0.05) in relation to the saline group. The bars were different in relation to the treatment ($F_{1,58}$= 3.18, p<0.01) time ($F_{2,18}$=3.32, p<0.05) and had time x treatment interaction ($F_{5,18}$= 4.23, p<0.05).

process of COPD potentiated the local inflammatory response caused by acute CFA hyperalgesia. This fact was repeated for T1, T3 and T24 hours.

The levels of IL-1β, IL-6 and TNF-α were higher in the plasma of animals with COPD within 24 hours, with significant differences ($p<0.05$), suggesting that within twenty-four hours after the induction of hyperalgesia by CFA, the pro-inflammatory cytokines originating from the systemic inflammatory process of COPD associated with the inflammatory mediators of the process caused by the CFA led to such an increase, in accordance with the results found and presented in figure 5.

For the CCI, the data is shown in figure 6, where it is possible to observe results similar to those of the CFA groups. The pressure threshold showed similar values in all groups to the baseline, with values around 42 g. The animals that underwent the CCI-Sham surgery, both with COPD and without the disease, presented values of pressure thresholds close to von Frey baseline values, showing that the CCI-Sham surgery did not change the pressure threshold of such animals.

However, for the animals that underwent CCI surgery, it is possible to observe a significant difference ($p<0.05$) in the pressure threshold when compared with the CCI-Sham animals. Conversely, the animals belonging to the COPD-CCI group, when compared to animals without the disease with CCI surgery, the result was statistically significant in the pressure threshold for the von Frey test, suggesting that the presence of inflammatory cytokines arising from COPD potentiates pressure threshold changes for CCI surgery.

When analyzing the inflammatory markers in CCI group (Figure 7), it is possible to observe a statistically significant difference ($p<0.05$) when comparing the levels of IL-1β, Il-6 and TNF-α of animals without COPD that underwent CCI surgery (saline), and animals with COPD that also underwent CCI surgery, at times T0 and T24. At time 0, this difference was less pronounced than at 24 hours, but with a significant result at both times.

This is consistent with what has been previously described, where animals with COPD had an even more evident response to the von Frey test when they had CCI surgery, compared to animals without the disease that underwent the same surgery, which corroborates the results shown in the figure 7, where plasma levels of markers were found in greater numbers in animals with COPD.

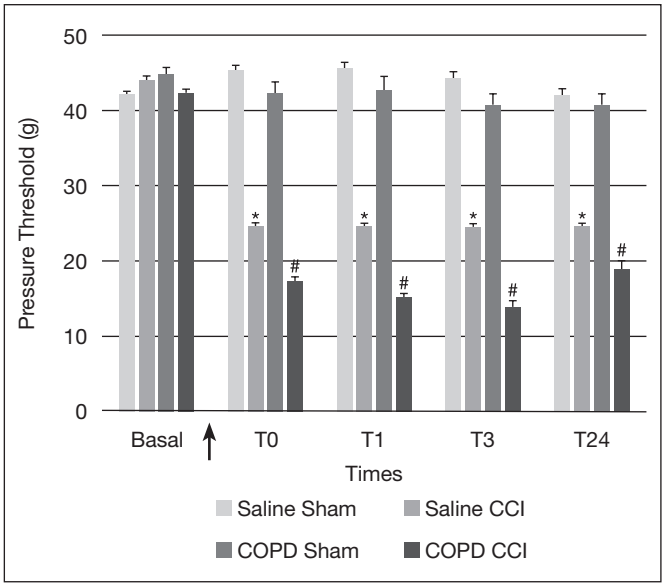

Figure 6. Pain assessment in CCI group, using von Frey test at times: baseline (before COPD induction), T0, T1, T3, and later T24 hours, performed 21 days after induction surgery.

*Significance ($p<0.05$) in relation to the saline group. # Different from all groups ($p<0,05$). In figure 6, the bars were different in relation to the treatment ($F_{3,20}$= 43.88, $p<0.01$) time ($F_{4,56}$=56.71, $p<0.05$) and had time x treatment interaction ($F_{12,56}$= 69.11, $p<0.05$).

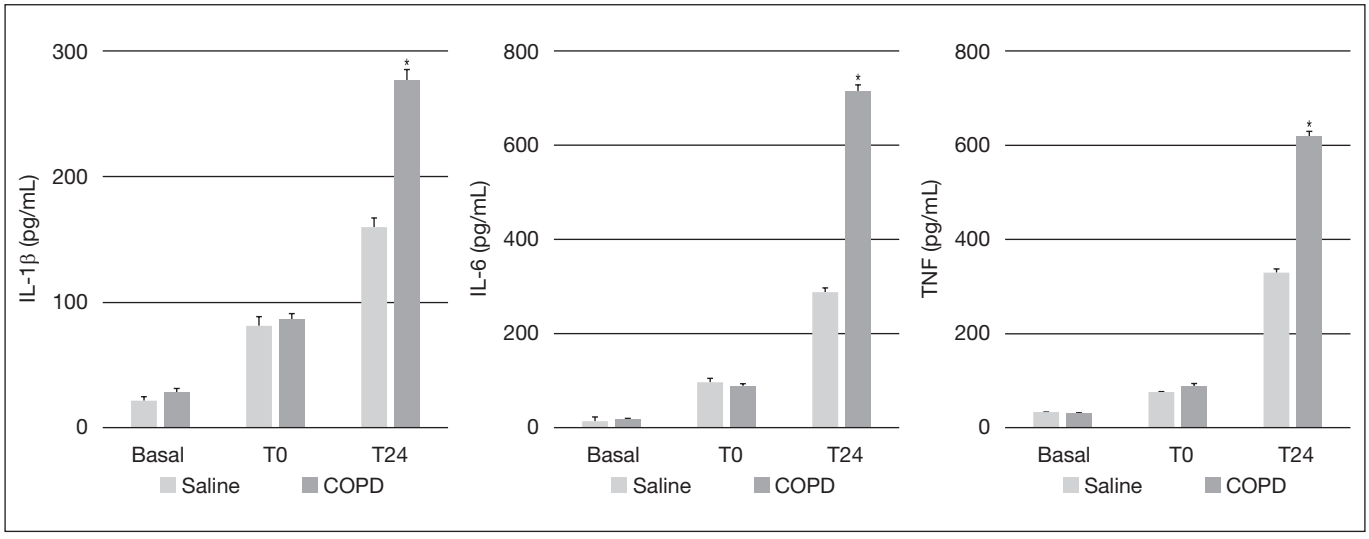

Figure 5. Evaluation of inflammatory markers in animals belonging to the inflammatory pain group, taking blood for analysis at: baseline (before COPD induction), T0, T1, T3, and later T24 hours, performed 21 days after induction surgery.

*Significance ($p<0.05$). In figure 5, the bars were different in relation to the treatment ($F_{1,58}$= 5.87, $p<0.01$) time ($F_{2,18}$=13.19, $p<0.05$) and had time x treatment interaction ($F_{5,18}$= 8.61, $p<0.05$).

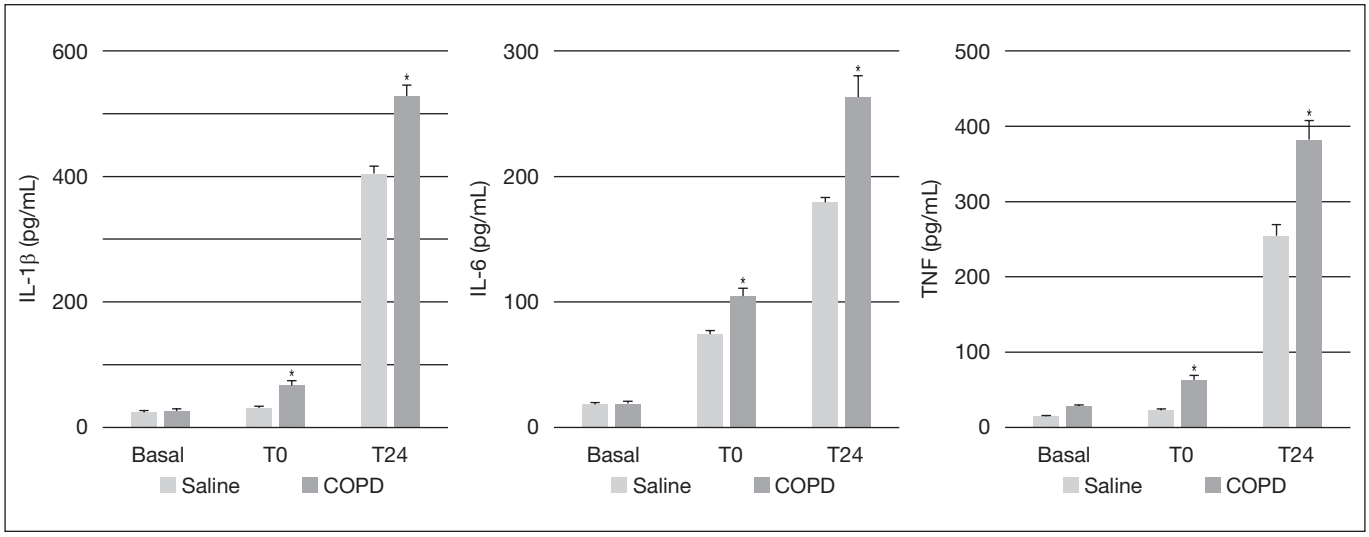

Figure 7. Evaluation of inflammatory markers in animals belonging to the neuropathic pain group, using blood withdrawal for later analysis at times: baseline (before COPD induction), T0, T1, T3, and later T24 hours, performed 21 days after induction surgery.

*Significance (p<0,05). In figure 7, the bars were different in relation to the treatment ($F_{1,58}$= 12.11, p<0.01) time ($F_{2,18}$=4.62, p<0.05) and had time x treatment interaction ($F_{5,18}$= 18.33 p<0.05).

DISCUSSION

Several methods are used as a model of emphysema induction, the model used in the present study was the instillation of a proteolytic enzyme, porcine pancreatic elastase, conversely there are other methods such as inhalation of cigarette smoke, instillation of proteolytic enzymes: plant protease (papain), human neutrophil elastase (HNE) and intratracheal lipopolysaccharide instillation[20].

A study[29] presented the first model using cigarette smoke in guinea pigs, where they were exposed to passive smoke for approximately 20 weeks. Moreover, it is believed that such a model portrays the moderate form of centrilobular COPD and the development of airway goblet cell metaplasia; thus it seems to be the model that most reliably portrays the alterations belonging to humans[30].

Conversely, the use of elastolytic enzymes is very well described, with the advantage of having a lower cost and faster onset of COPD, while exposure to cigarette smoke takes six months for the onset of the disease[31], which could make the experiment unfeasible. There is also a lack of standardization, and variations may occur, mainly regarding the system and protocol of exposure to cigarette smoke and the types of cigarettes used[29]. These facts were decisive for the choice of the COPD induction model in the present study.

The COPD model with tracheal instillation of proteolytic enzymes mimic the emphysema that occurs in human smokers based on the protease/anti-protease hypothesis[31]. That's because the protease/anti-protease imbalance of COPD was found after observations in smokers who had a high level of α 1-antitrypsin and an increased risk of developing the disease[32]. Therefore, several enzymes capable of degrading intact elastin have been injected into the lungs of animals to produce pulmonary emphysema[32,33].

COPD results in a series of important systemic effects, such as systemic inflammation, due to the presence of systemic oxidative stress, abnormal concentrations of circulating cytokines and activation of inflammatory cells[16,35]. Moreover, it is a lung disease with extrapulmonary manifestations, and inflammatory cells release substances such as elastase, collagenase, which modify the components of the extracellular matrix[16,35].

Several mediators involved in the inflammatory process are responsible for the vascular events of inflammation, and may stimulate local sensory neurons, which contribute to the activation of pain and/or nociception[36-38]. In the present study, it was possible to observe that animals with COPD had high levels of inflammatory markers, specifically IL-1β, IL-6 and TNF-α, in all groups.

It is already known that pro-inflammatory cytokines play a fundamental role in peripheral nociception[39], however, their relationship with the nociceptive threshold of individuals with COPD has not yet been described in any study. In the present study for the TF test, a method that also evaluates the tail withdrawal reflex under interference from central projections, the results showed that the rats belonging to the COPD group had a lower latency threshold than the control group. Moreover, the levels of IL-1β, IL-6 and TNF-α were increased, indicating that these markers contributed to the altered perception of the nociceptive threshold of animals with COPD.

The inflammatory process can generate pain not only due to the effects of leukocyte migration, it is believed that pro-inflammatory cytokines participate in the harmful process, and may originate from neuronal and glial immune cells, both in the peripheral nervous system (PNS) and in the central nervous system (CNS), where such molecules can trigger effects of chronic hyperexcitability leading to changes in the phenotypic expression of nociceptors, which results in abnormal processing and exacerbation of noxious signals[13].

In the persistence of the local aggressive stimulus, the glial cells which have self-regulatory properties lose the ability to maintain

biochemical homeostasis, causing the neuron to lose cell function and also causing programmed death[39]. Moreover, this fact may justify the findings found in the present study, which shows that the persistence of pro-inflammatory cytokines from the systemic inflammatory process of COPD could be responsible for the perception of altered noxious stimuli.

Corroborating with the justifications of this study, according to another research[39], the changes in the expression of ion channels, synapses and receptors of nerve cells are able to promote changes in neurotransmitters and neuromediators, allowing central and/or peripheral neurons to reach the threshold for depolarization earlier, generating ectopic discharges that amplify and activate neighboring cells where peripheral nociceptive stimuli can lead to classic synaptic sensitization, thus increasing the response of A and C afferent fibers[40,41].

To evaluate inflammatory pain, the present study used an increasing pressure test on the paw (von Frey test). The results found showed that there was a difference in the noxious response in rats with COPD that had CFA applied to the paw, when compared to control animals. Animals with COPD had an exacerbated response, suggesting that inflammatory markers that are elevated in animals with the disease could have contributed to such a response.

Accordingly with a study[42] on the inflammation induced by CFA, IL-6 seems to be of great importance since it has a participation in the induction of the inflammatory process and the generation of pain[43]. Stimulation by IL-6 on the afferent fibers of nociceptors may cause hyperalgesia during inflammation[41]. This was also the case in the present study, where the animals with COPD in the inflammatory pain group had a lower pressure threshold when compared to animals without the disease when both had CFA applied, suggesting that there was a somatization of pro-inflammatory cells contributing to the exacerbation of mechanical stimulus recognition. On the other hand, other markers were increased, not only IL-6, as described above, but also IL-1β and TNF-α.

Mediators released in the inflammatory response, referring to pain and noxious stimuli, are divided into intermediate hyperalgesic mediators that are released at the beginning and during inflammation, and final hyperalgesic mediators which directly interact with their specific receptors or nociceptors of primary afferent neurons, causing their sensitization/stimulation accordingly to a study[23], thus generating a reduction in the neuronal excitability threshold, an increase in the spontaneous activity of the nerve cell and an increase in the firing frequency in response to supra threshold stimuli[44]. Such evidence may justify the results found in the present study, where the COPD group presented alteration in the response to noxious stimuli, evidencing the presence of a systemic inflammation capable of altering the nociceptive threshold in rats.

The CCI model, that promotes alterations in the PNS through injury or ligation of peripheral nerves, results in chemotaxis of amebocytes to the site and, consequently, release of pro-inflammatory cytokines such as IL-1β and TNF-α[45]. This corroborates the findings of the present study, where the animals belonging to CCI-Sham group which did not undergo sciatic nerve constriction did not present differences regarding the pressure threshold. On the other hand, the animals which underwent the CCI surgery presented a lower pressure threshold. However, when comparing the animals without the disease that suffered CCI and with COPD ones, it was possible to observe that both had a change in the pressure threshold, but for those with the levels of markers already in greater quantity (animals with COPD) this threshold was changed more expressively, reaching lower values, suggesting that the cytokines present in COPD were able to amplify the response to the mechanical nociception stimulus.

TNF-α is one of the cytokines analyzed in the present study, it has the ability to initiate the inflammatory cascade, activating other cytokines, contributing to its effectiveness[46]; several cells can release this cytokine, including Schwann cells; its effects occur through interaction with the type I TNF receptor (sTNRF1), which has its expression increased after neuronal injury[47]. This inflammatory marker presented higher levels after COPD induction in all pain models, including phasic, inflammatory and neuropathic.

The set of both functional and structural modifications of nociceptors resulting from tissue injury is known as neuroplasticity. The effectiveness of synaptic conduction is described in the literature as sensitization, it can affect both the central nervous system and the peripheral nervous system of patients with chronic exposure to aggressive agents in the nervous system[38]. As in COPD, where the chronic exposure of systemic inflammatory markers such as IL-1β, IL-6 and TNF-α, were capable of altering the nociceptive perception. This fact was found in the present study, where animals which had emphysema induced by elastase. In addition to presenting increased levels of such markers, they also had different responses to the mechanical stimulus. Animals with COPD, in addition to presenting a lower latency threshold in TF, also presented a reduced pressure threshold when compared to animals without the disease who underwent the same procedures for both induction of hyperalgesia by CFA and by CCI.

The limitation found in the present study was the absence of a histological analysis of the animals' lungs in order to establish relationships between lung tissue impairment and nociceptive threshold.

CONCLUSION

Given the results presented, it was possible to verify that the animals with COPD had high levels of inflammatory markers, namely IL-1β, IL-6 and TNF-α. Animals with COPD presented altered responses to noxious stimuli, at the same time that cytokine levels were elevated, suggesting that, during this disease, in addition to promoting pulmonary manifestations, the systemic inflammatory process arising from COPD interferes with the perception of nociceptive stimuli.

However, it was possible to observe a change only in the presence of stimulation of both the CFA and the CCI, indicating that in the presence of a local inflammatory process or in a neuropathy, systemic cytokines from COPD would add up, causing an exacerbation of the nociceptive stimulus.

AUTHORS' CONTRIBUTIONS

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Statistical Analysis, Data Collection, Conceptualization, Project Management, Research, Methodology, Writing – Preparation of the Original, Writing - Review and Editing, Validation, Visualization.
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Statistical analysis, Software, Supervision, Validation.
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Project Management, Supervision.
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Data Collection, Methodology.
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