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Original Article

Effects of Melatonin on Airway Inflammation in Experimental Models of Asthma, COPD and the Asthma-COPD Overlap

Efeitos da Melatonina na inflamação de vias aéreas em modelos experimentais de asma, DPOC e de sobreposição Asma-DPOC

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ABSTRACT: *Background and Objectives:* According to previous studies, melatonin, an indolamine, may possibly reduce dyspnea in patients with asthma or COPD. Additionally, it has also been proven, through experimental studies, that the molecule possibly plays an anti-inflammatory role, because it might inhibit transcription of NFk-B in experimental models of asthma and COPD. This study aims to investigate the effects of melatonin in experimental models of asthma, COPD and Asthma-COPD Overlap (OCP). *Methods*: 64 mice were divided into 8 groups in order to induce COPD (group "ALS"), asthma ("OVA") or OC ("ACO"). The control group ("SAL") received saline. The treatment groups ("+MEL") were submitted to both disease protocols and also received Melatonin (intraperitoneally). After the protocols, the exhaled nitric oxide (Eno) and the total and differential cells of the bronchoalveolar lavage fluid were evaluated. *Results*: OVA+MEL (11.3±1.65ppb) showed reduction in Eno compared to OVA (24.17±4.30ppb). In the analysis of the bronchoalveolar lavage fluid cells, OVA+MEL ($11.3\pm1.65x10^4$ cells/mL) and ACO+MEL ($2.17\pm0.63x10^4$ cells/mL) showed a reduction in the amount of total cells compared to OVA ($25.70\pm4.59x10^4$ cells/mL) and ACO ($14.33\pm3.11x10^4$ cells/mL), respectively. There was a decrease in eosinophil count in OVA+MEL ($5.37\pm1.41x10^4$ cells/mL) and ACO+MEL ($0.87\pm0.36x10^4$ cells/mL) compared to OVA ($18.67\pm4.01x10^4$ cells/mL) and ACO ($1.45\pm0.41x10^4$ cells/mL), respectively. Additionally, the number of lymphocytes decreased in OVA + MEL ($1.00\pm0.24x10^4$ céls./mL) compared to OVA ($3.94\pm1.15x10^4$ cells/mL). The number of macrophages also decreased in OVA + MEL ($3.09\pm0.39x10^4$ cells/mL) and ACO + MEL ($1.10\pm0.27x10^4$ cells/mL) compared to OVA ($7.26\pm1.93x10^4$ cells/mL) and ACO ($6.41\pm1.54x10^4$ cells/mL), respectively. There was no difference in the comparison of neutrophil counts in the different groups analyzed. *Conclusion:* Treatment with melatonin was, in experimental models of asthma and ACO, effective in reducing pro-inflammatory parameters, and may play an important role in the control of such pathologies. In addition, treatment with melatonin in experimental models of elastase-induced lung injury was not effective in controlling pro-inflammatory parameters in bronchoalveolar lavage fluid, and further studies are needed to understand the mechanisms of action of melatonin in lung tissue and airways.

Keywords: Melatonin; Inflammation; Remodeling; Asthma; COPD; ACO.

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RESUMO: *Justificativa e Objetivos:* De acordo com estudos anteriores, a melatonina, uma indolamina, pode possivelmente reduzir a dispneia em pacientes que apresentam asma ou DPOC. Adicionalmente, foi provado igualmente, através dos estudos experimentais, que a molécula joga possivelmente um papel antiinflamatório, porque pôde inibir a transcrição de NFk-B em modelos experimentais da asma e do COPD. Este estudo tem como objetivo investigar os efeitos da melatonina em modelos experimentais de asma, DPOC e Sobreposição da Asma-DPOC (ACO). *Métodos*: 64 camundongos foram divididos em 8 grupos, a fim de induzir DPOC (grupo "ELA"), asma ("OVA") ou ACO ("ACO"). O grupo controle ("SAL") recebeu solução salina. Os grupos de tratamento ("+MEL") foram submetidos aos dois protocolos de doença e também receberam Melatonina (intraperitonealmente). Após os protocolos, foram avaliados o óxido nítrico exalado (Eno), bem como as células totais e diferenciais do fluido do lavado broncoalveolar. *Resultados:* OVA+MEL (11.3±1.65ppb) apresentou redução em eNO em comparação a OVA (24.17±4.30ppb). Além disso, OVA+MEL (11.3±1.65x10^4 céls./mL) e ACO+MEL (2.17.±0.63x10^4 céls./mL) apresentaram redução na quantidade de células totais em comparação a OVA (25.70±4.59x10^4 céls./mL) e ACO (14.33±3.11x10^4 céls./mL), respectivamente. Houve queda na contagem de eosinófilos em OVA+MEL (5.37±1.41x10^4 céls./mL) e em ACO+MEL (0.87±0.36x10^4 céls./mL) em comparação a OVA (18.67±4.01x10^4 céls./mL) e ACO (1.45±0.41x10^4 céls./mL), respectivamente. Adicionalmente, o número de linfócitos apresentou redução em OVA+MEL (1.00±0.24x10^4 céls./mL) em comparação a OVA (3.94±1.15x10^4 céls./mL). O número de macrófagos também apresentou redução em OVA+MEL (3.09±0.39x10^4 céls./mL) e em ACO+MEL (1.10±0.27x10^4 céls./mL) em comparação a OVA (7.26±1.93x10^4 céls./mL) e ACO (6.41±1.54x10^4 céls./mL), respectivamente ($p<0.05$ para todas as comparações). Não houve diferença na comparação da contagem de neutrófilos nos diferentes grupos analisados. *Conclusão:* O tratamento com melatonina mostrou-se, em modelo experimental de asma e ACO, efetivo na redução de parâmetros pró-inflamatórios, podendo representar um papel importante no controle de tais patologias. Além disso, o tratamento com melatonina em modelos experimentais de lesão pulmonar induzida por elastase não se mostrou eficaz no controle de parâmetros pró-inflamatórios no fluido do lavado broncoalveolar, sendo necessários mais estudos para o entendimento dos mecanismos de ação da melatonina no tecido pulmonar e nas vias aéreas.

Palavras-chave: Melatonina; Inflamação; Remodelamento; Asma; DPOC; ACO.

INTRODUÇÃO

Asthma

Asthma is a chronic inflammatory disease characterized by hyperresponsiveness (HR) of the lower airways and by variable limitation to airflow, reversible spontaneously or with treatment, manifesting itself clinically by recurrent episodes of wheezing, dyspnea, chest tightness and coughing, particularly at night and in the morning upon awakening. It results from an interaction between genetics, environmental exposure and other specific factors leading to the development and maintenance of symptoms[1].

The main physiopathological characteristic of asthma is bronchial inflammation, resulting from a broad and complex spectrum of interactions between inflammatory cells, mediators and structural cells of the airways[2,3]. The allergic inflammatory response is initiated by the interaction of environmental allergens with cells that have the function of presenting these allergens to the immune system, more specifically Th2 lymphocytes. These, in turn, produce cytokines responsible for the initiation and maintenance of the inflammatory process. IL-4 plays an important role in increasing the production of allergen-specific IgE antibodies[1,2,4].

Several inflammatory mediators are released by mast cells (histamine, leukotrienes, tryptase and prostaglandins), by macrophages (tumor necrosis factor - TNF-alpha, IL-6, nitric oxide), by T lymphocytes (IL-2, IL-3, IL-4, IL-5), eosinophils (major basic protein, ECP, EPO, lipid mediators and cytokines), neutrophils (elastase) and epithelial cells (endothelin-1, lipid mediators, nitric oxide). Through such inflammatory response, damage and changes in epithelial integrity is produced. Furthermore, changes in vascular permeability, hypersecretion of mucus, changes in mucociliary function and increased reactivity of airway smooth muscle is present[3]. These mediators can also reach the ciliated epithelium, causing damage and rupture. As a consequence, epithelial cells and myofibroblasts, located below the epithelium, proliferate and initiate the interstitial deposition of collagen in the reticular lamina of the basement membrane, which explains the apparent thickening of this membrane and the irreversible lesions that may occur in some patients with asthma. Other changes, including hypertrophy and smooth muscle hyperplasia, increase in the number of goblet cells, increase in submucosal glands and change in deposition and degradation of extracellular matrix components, are constituents of the remodeling that interferes with the architecture of the airway, leading to the irreversibility of obstruction observed in some patients[4].

Chronic obstructive pulmonary disease

Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death among chronic non-communicable diseases in Brazil, and its prevalence varies according to region and smoking rate[5]. COPD is preventable and treatable. It is characterized by the presence of chronic, persistent airflow obstruction. This process is usually progressive and is associated with abnormal inflammatory response of the lungs and with inhalation of toxic particles and gases. Although COPD affects the lungs, it also produces significant systemic consequences. The chronic inflammatory process of the airways is a very relevant feature in the pathophysiology of COPD. Chronic inflammation causes structural changes and decreased

bronchial lumen. The destruction of the pulmonary parenchyma leads to the loss of alveolar coupling in the small airways and decreases the elastic collection of the lung. These changes decrease the ability of the airways to remain open during expiration[5,6].

Inflammation affects large and small airways, where we find hyperplasia of goblet cells, increased mucous glands, infiltration of neutrophils, macrophages and CD8+ lymphocytes in the large airways, especially in the more advanced stages of COPD. Inflammatory infiltrate is also found in the smaller airways, with a predominance of macrophages in the early stages of the disease[7]. The destruction of lung tissue results from the imbalance between proteinases and antiproteinases. Evidence supports the hypothesis that inhalation of cigarette smoke induces an increase in the number of neutrophils and macrophages, which release enzymes such as cathepsins B, K, L and S, neutrophilic elastase, proteinase 3, matrix metalloproteinase (MMP) 9 and 12. These enzymes are not fully inhibited by antiproteinases, leading to destruction of the pulmonary connective tissue[5].

Bronchial remodeling is characterized by increased deposition of extracellular matrix proteins in the airways, which leads to subepithelial fibrosis, hypertrophy and smooth muscle hyperplasia, as well as hyperplasia of the submucosal glands, which results in the thickening of the bronchial wall and the smaller bronchial caliber. Such changes are also observed in patients with bronchitis and in experimental studies, suggesting that bronchial remodeling occurs due to the release of several inflammatory factors, cell growth and cytokine release[8]. Thus, better understanding of the pathophysiology of COPD and its proper treatment are among the preventive strategies of the disease.

Asthma-COPD Overlap (ACO)

Asthma and COPD already have diagnostic criteria and widely discussed and defined pathophysiological mechanisms. Currently, a variant of these diseases that has characteristics of both has been studied and called Asthma-COPD Overlap (ACO)[4,9]. The association Asma-DPOC is an airway disease characterized by fixed obstruction to airflow with characteristics common to both asthma and COPD, but a more specific definition could not be established, due to the lack of scientific evidence on the subject[4]. Its prevalence is little known because it lacks clear definition.

Melatonin

Melatonin was first isolated and characterized from the bovine cavity by dermatologist Aaron Lerner, in 1958[10]. It is the main hormone secreted by the pineal gland. Secondary sources are the retina, intestine, skin, platelets and bone marrow and probably other structures, but their systemic contribution is negligible[11].

Melatonin presents high solubility in lipids and water, which facilitates the passage through cell membranes[12]. Circulating melatonin can reach all tissues of the body and is able to cross the blood-brain barrier to modulate brain activity[13]. In recent years, melatonin has been used both for physiological effects (such as sleep cycle control and seasonal adaptation) and for its therapeutic activities in cardiovascular and bone metabolisms, renal functions, gastrointestinal system and anticancer effects.

Studies indicate that intraperitoneal administration of melatonin at 10mg/kg significantly inhibits the expression of NF-κB and negatively regulates the activity of inducible NO synthesis in lung tissues and also significantly reduces the production of NO in bronchoalveolar lavage in an experimental animal model of bronchial asthma[14]. The authors concluded that melatonin may decrease hyperresponsiveness and airway inflammation in the ovalbumin model of bronchial asthma. The same authors found, in a later study, that melatonin significantly inhibits the expression of connective tissue growth factor and reduces the area of mucus in the ovalbumin model of asthmatic mice, with activity similar to that exerted by dexamethasone[15].

In addition, a double-blind, randomized, placebo-controlled study in asthmatic adults demonstrated that the oral supplement of 3mg of melatonin orally for 28 days improved the subjective sleep quality in patients with mild or moderate asthma compared to the placebo group[16].

Justification

Currently it is known that obstructive pulmonary diseases are an important health issue and are among the leading causes of death worldwide. Epidemiological data show an increase in the number of deaths caused by COPD, with an increase of 340% in the 20-year period[5]. It should be noted that obstructive diseases are responsible for high hospital costs, being associated with several comorbidities and generate an important impact on the functional life of the individual. Although attempts have been made to treat asthma and chronic obstructive pulmonary disease, this area of research still remains a field that deserves much attention especially for the most severe conditions.

Melatonin has the ability to mobilize the mechanisms of antioxidant enzymes of different tissues, in addition to regulating smooth muscle tone and influencing the immune response, being highly efficient in eliminating free radicals, with antioxidant properties. There is little evidence on the response of melatonin in asthma and COPD and there is no evidence in experimental studies on Asthma-COPD overlap syndrome. Thus, Melatonin could possibly play a role in controlling the pathophysiological mechanisms of the aforementioned diseases.

OBJECTIVES

This study aims to evaluate whether melatonin modulates pulmonary inflammation (assessed by the

bronchoalveolar lavage fluid cells count), and the amount of exhaled nitric oxide in experimental models of asthma, COPD and ACO.

MATERIALS AND METHODS

Animals

This study was approved by the Research Ethics Committee (CEUA - FMUSP). 64 male BALB/c mice were obtained from the central vivarium. The animals were between 6 and 8 weeks old when leaving the central vivarium and underwent a period of setting in the laboratory vivarium of 2 weeks.

The animals were fed ad libitum and were kept in a light-dark cycle of 12 hours, under controlled conditions of room temperature (22ºC), humidity and noise, by trained professionals, responsible for the care of the animals during the period of their stay in the vivarium.

The necessary care received by the animals is in accordance with the "Guide of care and use of laboratory animals" published by the "US National Institutes of Health" (NIH Publication No 85-23, revised in 1996) and the Arouca Law (Law 11.794 of October 8, 2008).

The mice were randomly divided into 8 groups (n = 8) according to the exposure protocol to which they were submitted:

The experimental groups were:

A) SAL (control group - received intratracheal instillation of saline solution (50 µl)). (n=8)

B) OVA (asthma model - sensitized with ovalbumin) (n=8);

C) SAL+MEL (received intratracheal instillation of saline solution and was treated with intraperitoneal instillation of melatonin) (n=8);

D) OVA + Melatonin (sensitized with ovalbumin and treated with intraperitoneal instillation of melatonin) (n=8);

E) ELA (COPD model - received intratracheal instillation of porcine pancreatic elastase (EPP) (EMD Chemicals, San Diego, CA)) (n=8);

F) Elastase + Melatonin (received intratracheal instillation of porcine pancreatic elastase and also treated with intraperitoneal instillation of melatonin (n=8);

G) ACO (asthma-COPD overlap model - sensitized with ovalbumin and also received intratracheal instillation of porcine pancreatic elastase) (n=8);

H) ACO + Melatonin (sensitized with ovalbumin, received intratracheal instillation of porcine pancreatic elastase (EPP) (EMD Chemicals, San Diego, CA) and also treated with intraperitoneal instillation of melatonin). (n=8).

On the 28th day of the protocols, the following analyses were carried out in all groups:

(i) exhaled nitric oxide measurement;

(ii) quantification of the total number of cells, macrophages, eosinophils, neutrophils and lymphocytes in bronchoalveolar lavage fluid (BALF);

Details of the protocols

Experimental model of Asthma

The protocol of sensitization and induction of pulmonary inflammation by ovalbumin lasted 28 days. The mice received 50 mg solution of ovalbumin (Sigma - Aldrich) and 6 mg of Aluminum Hydroxide - Alumen (Pepsamar, Sanofi-Synthelabo S.A., Rio de Janeiro, Brazil) intraperitoneally (i.p.) on days 1 and 14. On days 21, 23, 25 and 27 the animals were placed in an acrylic exposure box coupled to an ultrasonic nebulizer (US - 1000, ICEL, São Paulo, Brazil) and submitted to inhalation of OVA aerosol diluted in 0.9% NaCl (saline) at a concentration of 10 mg/ml (1%). The time the animals were in contact with the aerosol was 30 minutes. At the same time, the control group received saline solution (NaCl 0.9%) and aluminum hydroxide (Alúmen) (6 mg) intraperitoneally (i.p.) and in were exposed 0.9% NaCl aerosol for 30 minutes[17].

Experimental model of COPD

The mice were anesthetized with isoflurane and received intratracheal instillation of porcine pancreatic elastase (EPP) on day 21 of the experimental protocol (EMD Chemicals, San Diego, CA) - at the dose and concentration of 25 U EPP/100 g body weight dissolved in 40 µl saline solution[18].

Experimental Model of ACOS

The mice of this experimental group were submitted to the two experimental protocols described above (asthma model and elastase-induced lung injury), following the dose and date protocols[18,19].

Experimental Model of Melatonin

The mice were treated intraperitoneally with Melatonin [15mg/kg (0.45/day)], from day 22 to 27[20].

Evaluation of Exhaled Nitric Oxide

During mechanical ventilation, gas was collected in the expiratory portion of the ventilator through a balloon impervious to the NO (Mylar Bag, Sievers, Instruments Inc., Boulder, CO, USA) for 10 minutes. After the end of the collection period, the balloons were sealed for further analysis. Nitric oxide was then measured by chemiluminescence through a rapid response analyzer (280 NOA - Nitric Oxide Analyzer - Sievers Instruments *Inc., Boulder*, CO, USA). The average concentration of NOex was recorded in parts per billion (ppb), as an index of NO concentration in exhaled air.

Evaluation of Bronchoalveolar Lavage Fluid (BALF)

BALF was produced through an infusion of 0.5 ml of saline for three consecutive times (total volume = 1.5ml), by tracheal cannula, with the use of a syringe. The volume

recovered was centrifuged at 1000 rpm, at 5ºC, for 10 minutes, with an average recovery of 80%. The cell button was resuspended in 200 µl of saline. The total cell count was performed by optical microscopy with the Neubauer hemocytometer (400x). For differential counting, 100 µl of the LBA was cytocentrifuged at 450 rpm for 6 minutes and after drying the blade was stained by the Diff-Quick technique. The differential cell count was determined from 300 leukocytes/lamina and the differentiation followed the cytological criteria for differentiation of neutrophils, eosinophils, lymphocytes and macrophages with the aid of an optical microscope with immersion objective (1000X)[21].

Disposal of the carcasses

The carcasses of the animals were placed in white garbage bags, identified with standard labels and stored in a refrigerator. The bags were then carried out by employees of the hospital technical cleaning company and the waste transported to the waste shelter, for daily overnight collection for treatment and final disposal, after the issuance (by the responsible veterinarian) of the report and of the movement document.

RESULTS

All data from the groups were analyzed using unidirectional analysis of variance (ANOVA). The parametric data were presented as mean standard error (SE), and were compared using the Holm-Sidak test, being presented in the form of bars. All analyses were performed using SigmaPlot 11.0 software (Systat Software, SPSS Inc., USA). A p-value <0.05 was considered statistically significant.

The results obtained in all parameters of the SAL-MEL group, when compared with the SAL group (control), did not show any difference. Furthermore, the comparison between SAL-MEL and other groups presented the same results as the comparison between SAL and disease and treatment groups. Therefore, the results from SAL and SAL-MEL were clustered and presented as one in the following analysis.

Analysis of total cells of bronchoalveolar lavage fluid (BALF)

Figure 1 shows the total cell count present in the bronchoalveolar lavage fluid (104 cells/mL) in the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups. There was an increase in total cell count of OVA, OVA-MEL and ACO compared to the SAL group ($p<0.05$ for all comparisons). There was a decrease in total cell count in the OVA-MEL group compared to the OVA-MEL group ($p<0.05$). There was a decrease in total cell count of the ACO-MEL group compared to the ACO group and compared to the OVA-MEL group ($p<0.05$ for both comparisons). There was no difference between the groups SAL, ACO-MEL, ELA and ELA-MEL.

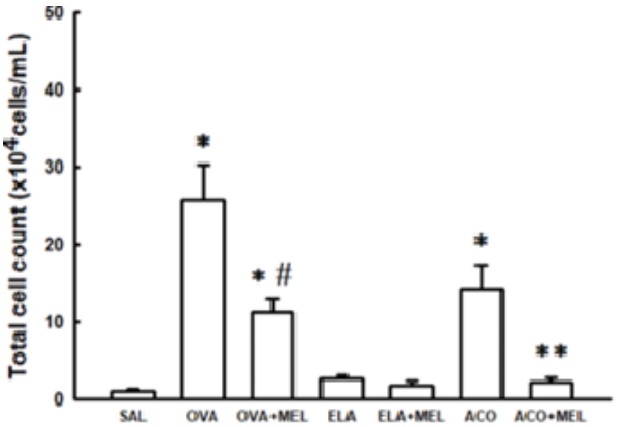

Figure 1: Graph of total cell count of SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups; *p<0.05 for OVA, OVA-MEL and ACO, compared to SAL group; #p<0.05 for OVA-MEL, compared to OVA group; **p<0,05 for ACO-MEL compared to ACO group; ¯p<0,05 for ACO-MEL compared to OVA-MEL group

Analysis of bronchoalveolar lavage fluid - differential cells

Eosinophils

Figure 2 shows the eosinophil count present in the bronchoalveolar lavage fluid (104 cells/mL) in the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups. There was an increase in eosinophil count in the OVA, OVA-MEL and ACO group compared to the SAL group ($p<0.05$ for both comparisons). There was a decrease in eosinophil count in the OVA-MEL group compared to the OVA group ($p<0.05$). There was a reduction in the eosinophil count in the ACO-MEL group compared to the ACO group ($p<0.05$). The eosinophil count showed no difference between the groups SAL, ACO-MEL, ELA and ELA-MEL.

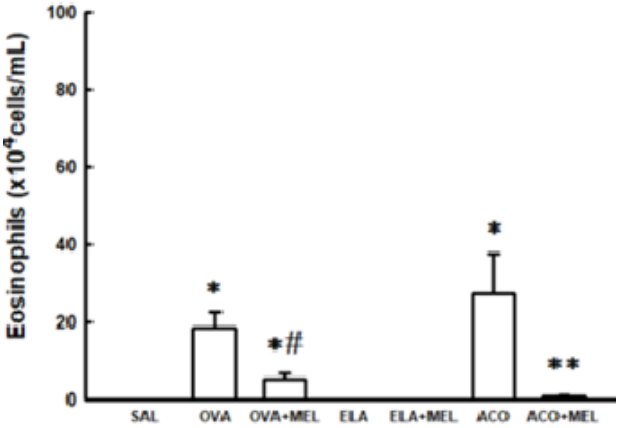

Figure 2: Graph of eosinophil count of the groups of SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL; *p<0.05 for OVA, OVA-MEL and ACO, compared to the SAL group; #p<0.05 for OVA-MEL, compared to the OVA group; **p<0,05 for ACO-MEL compared to ACO group

Lymphocytes

Figure 3 shows the lymphocyte count present in the bronchoalveolar lavage fluid (104 cells/mL) in the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups. There was an increase in lymphocyte count in the OVA group compared to the SAL group ($p<0.05$). There was a reduction in lymphocyte count in the OVA-MEL group compared to the OVA group ($p<0.05$). There was no difference in the number of lymphocytes between the SAL, ELA, ELA-MEL and ACO and ACO-MEL groups.

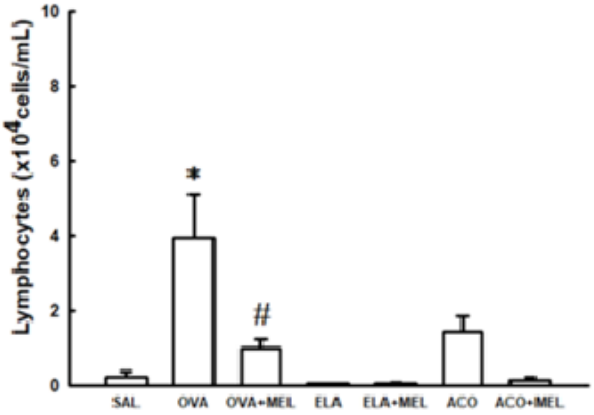

Figure 3: Graph of lymphocyte count of SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups; *p<0,05 for OVA compared to SAL group; #p<0,05 for OVA-MEL compared to OVA group

Macrophages

Figure 4 shows the count of macrophages present in the bronchoalveolar lavage fluid (104 cells/mL) in the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups. There was an increase in counting in the OVA and ACO groups compared to the SAL group ($p<0.05$). There was a reduction in counting in the ACO-MEL group in relation to ACO ($p<0.05$). There was no difference between the groups SAL, OVA-MEL, ELA, ELA-MEL and ACO-MEL. There was no difference between the groups OVA and OVA-MEL.

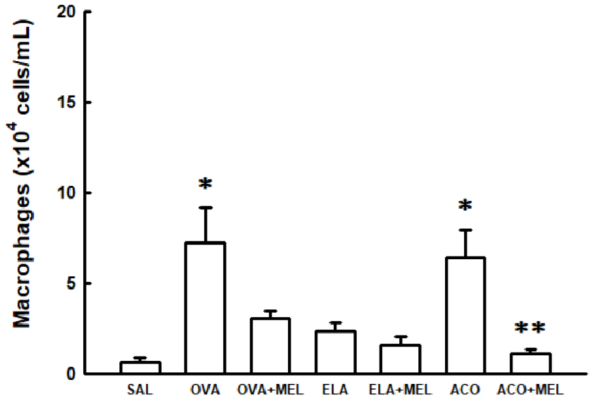

Figure 4: Graph of the macrophage count of the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups; *p<0,05 for OVA and ACO compared to the SAL group; **p<0,05 for ACO-MEL compared to the ACO group

Neutrophils

Figure 5 shows the neutrophil count present in the bronchoalveolar lavage fluid in the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups. There were no differences between the groups analyzed.

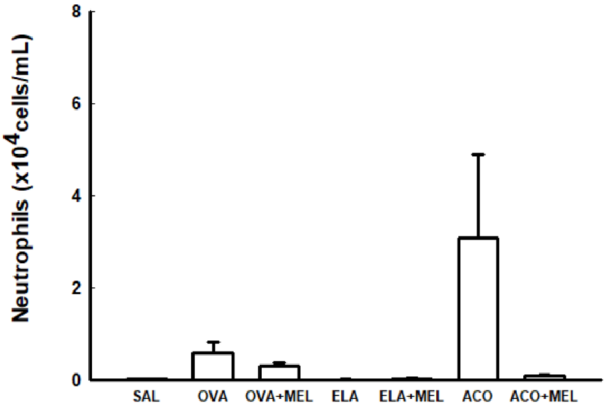

Figure 5: Graph of the neutrophil count of the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups; there was no difference between the groups analyzed

Exhaled nitric oxide

Figure 6 demonstrates the quantification of exhaled nitric oxide (NO, as ppb) in the SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL groups. There was an increase in the amount in the OVA group, compared to the SAL group ($p<0.05$). There was a decrease in the amount in the OVA-MEL group compared to the OVA group ($p<0.05$). There was no difference in the amount of exhaled NO between the groups SAL, OVA-MEL, ELA, ELA-MEL, ACO and ACO-MEL.

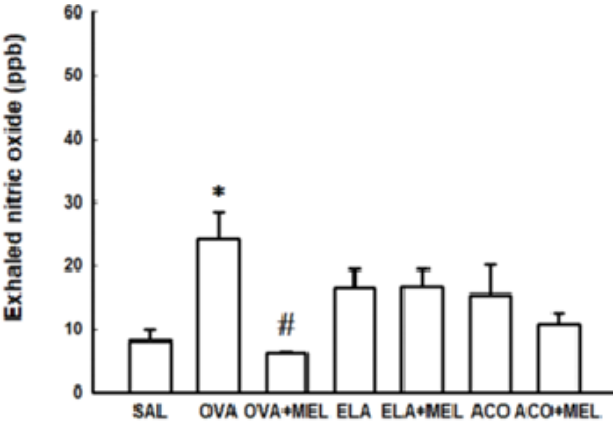

Figure 6: Graph of the quantification of exhaled nitric oxide of the groups SAL, OVA, OVA-MEL, ELA, ELA-MEL, ACO, ACO-MEL; *p<0,05 for OVA, when compared to the SAL group; #p<0,05 for OVA-MEL, when compared to the OVA group

DISCUSSION

Inflammatory response evaluated by cellular analysis of bronchoalveolar lavage fluid

We observed that the animals of the experimental asthma model using ovalbumin showed an increase in the number of total cells, macrophages, eosinophils and lymphocytes, as expected in the pathophysiology of this disease. Melatonin treatment reduced the number of total cells, eosinophils and lymphocytes in this experimental asthma model.

In the model of pulmonary injury induced by elastase (ELA and ELA-MEL) there were no significant changes in the cells of bronchoalveolar lavage.

When evaluating the animals of the ACO model, we observed that there was an increase in the number of total cells, macrophages and eosinophils. We also noticed that the response observed in the ACO animals was similar to that observed in the experimental asthma model with the exception of the lymphocyte response. To better understand this data, it will be necessary to evaluate the lymphocyte response profile (Th1, Th2 and Th17). We noticed that the treatment with melatonin reduced the number of total cells, macrophages and eosinophils, reinforcing the relationship between the pathophysiology of asthma and ACO.

As discussed in the ACO model, there was an increase of total cells represented by macrophages, eosinophils and lymphocytes as well as the one observed in the experimental asthma model, demonstrating once again the relationship between ACO and Asthma.

Exhaled nitric oxide

Animals of the experimental asthma model using ovalbumin showed an increase in exhaled NO and treatment with melatonin reduced this parameter. This result corroborates the findings of inflammatory response observed in these animals in bronchoalveolar lavage fluid.

In the model of pulmonary injury induced by elastase (ELA and ELA-MEL) there were no changes in exhaled NO. Also, there was no difference between the groups ACO and ACO+MEL, there was no differin exhaled NO.

NO is endogenously synthesized by one of three isoforms of NOS, with different activities, two constitutive (Enos, derived from the endothelium; and nnos, derived from neurons) and one induced NOS (Inos)[22,23]. Constitutive isoforms are involved in vasodilation and bronchodilation. The Inos is stimulated by many proinflammatory cytokines and is expressed in some types of inflammatory cell[24,25]. The NO derived from the enzyme Nnos has a beneficial effect on asthma by causing relaxation in the bronchial smooth muscles, being present in the nerves of the NANC system. However, NO derived from the enzyme Enos can lead to vasodilation in the arterioles, with consequent plasma extravasation and edema. The large amount of NO from the Inos enzyme can result in vasodilation, plasma extravasation, increased mucus secretion and indirect activation of Th1/Th2 cells contributing to the signs and symptoms of the disease[4]. A reduction in the production of NO, seen in the OVA-MEL group, in comparison to OVA, can therefore lead to a reduction in mucus secretion and also in deactivation of Th1/Th2 cells.

Furthermore, the reaction of NO with free radicals such as oxygen produces nitrogen reactive compounds that exert physiological functions and are important for the destruction of microorganisms[23,25]. However, the inflammatory cells of asthmatics have an increased ability to generate free radicals when compared to controls, which subsequently contribute to high concentrations of nitrogen reactive compounds, which can cause deleterious effects[26]. When the airways are exposed to oxidative stress, peroxynitrite (ONOO-), one of the potent oxidants formed by the reaction between NO and oxygen, is responsible for several changes, it is worth mentioning the damage to DNA, stimulation of the degradation of lipids, proteins and carbohydrates, which can alter cellular functions and potentiate inflammatory responses[26]. According to a previous study, melatonin possibly exerts an antioxidant effect[27], as also seen in the OVA+MEL group. Controlling the expression of NO may play a significant role in reducing the oxidative stress upon the airways and lung parenchyma.

CONCLUSÃO

Melatonin plays a possible anti-inflammatory role and control of bronchial hyperresponsiveness in experimental models of asthma and OC, attenuating mainly the inflammatory response of eosinophilic component. Melatonin was indifferent in the inflammatory control of experimental models of elastase-induced lung injury. Further studies are needed to understand the exact mechanisms of action of the molecule in airways and lung parenchyma.

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