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Modulation of the endogenous Annexin A1 in a cigarette smoke cessation model: Potential therapeutic target in reversing the damage caused by smoking?

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ABSTRACT

Background: Smoking cessation may help in the reversal of inflammation and damage caused by smoking. The endogenous annexin A1 (AnxA1) protein has anti-inflammatory effects which instigates the understanding of its role in the attenuation of inflammatory processes caused by smoking.
Material and methods: Wistar rats were exposed to cigarette smoke for 8 weeks. After the exposure period, one of the groups remained other 8 weeks in the absence of smoke. Animals not exposed to smoke were used as control. Blood, trachea and lungs were obtained for histopathological, immunohistochemical and biochemical analyses.
Results: Loss of cilia of the tracheal lining epithelium was found by smoke exposure, but smoking cessation led to recovery of the tracheal epithelium. Similarly, chronically exposed-to-smoke animals showed increased lymphocytes and macrophages in bronchoalveolar lavage and higher levels of glucose and gamma-GT in their blood. Reduction of lymphocytes, glucose and gamma-GT occurred after smoking cessation. In addition, IL-1β, IL-6, IL-10, TNF-α and MCP-1 levels were elevated by smoke exposure. Smoking cessation significantly reduced the levels of IL-1β, IL-6 and MCP-1 but increased the IL-10 concentration. Numerous mast cells and macrophages were observed in the lung of chronically exposed-to-smoke animals with reduction by smoking cigarette abstinence. AnxA1 increased expression and concomitant NF-κB reduction were found in the smoking cessation group.
Conclusion: Our results showed that cigarette abstinence promoted partial recovery of the inflammatory process. The attenuation of the inflammatory profile may be associated with the overexpression of AnxA1 protein.

1. Introduction

Smoking is an important global health problem and is strongly associated with the development of chronic obstructive pulmonary disease (COPD), a serious health condition characterized by progressive airflow limitation and estimated to be the third leading cause of death in 2020 [1–4].

Inflammation induced by irritants contained in cigarette smoke triggers the histopathological changes of COPD, including hypersecretion of mucus and destruction of alveolar walls. The accumulation of inflammatory cells such as macrophages, neutrophils, lymphocytes and mast cells and the consequent release of several chemical mediators are

Abbreviations: AnxA1, Annexin A1; BAL, bronchoalveolar lavage; C, control group; COPD, Chronic Obstructive Pulmonary Disease; COX-2, cyclooxygenase-2; CS, chronic smoker group; Gamma, GT gamma-glutamyltransferase; (HOMA-IR), insulin resistance index of homeostatic model assessment; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; MCP-1, monocyte chemoattractant protein-1; SC, smoking cessation group; TNF-α, tumor necrosis factor-α

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responsible for the progression of the disease [2,3,5–7].

An important strategy for reducing lung function decline and relieving the inflammatory process is smoking cessation. However, investigations have shown that the inflammatory process is not resolved by smoking cessation in patients who have already started COPD [8–10]. Animal smoking cessation models also showed the persistence of innate inflammatory response and maintenance of the disease with accumulation of natural killer (NK) lymphocytes and macrophages as well as increased levels of interleukin (IL)-17A and serum amyloid A (SAA), as markers of persistence [11]. In mice the pulmonary inflammation was partially reversed by smoking cessation, however, macrophage numbers and matrix metalloproteinase (MMP)-12 expression remained increased [12].

Given the central role played by inflammation in COPD, understanding the mediators involved in resolving the inflammatory process associated with this disease is critical to the development of innovative anti-inflammatory therapies [3,7,13]. In this context, the Annexin A1 protein (AnxA1), the first characterized member of the annexin superfamily [14–16] deserves attention. The AnxA1 can bind to the phospholipid bilayer of cell membranes or to G-protein coupled receptor as formyl peptide receptor 2 (FPR2) to perform its effects [7,16]. Both the protein and its mimetic peptide, Ac2-26, showed strong anti-inflammatory profiles in many animal models of acute and chronic inflammation, including different lung conditions [17–21].

In the context of pulmonary inflammation, the importance of AnxA1 as mediator of the inflammatory process was demonstrated in different models. AnxA1 knockout mice exposed to silica showed altered lung functions and exacerbated inflammatory and fibrotic responses [22]. In animals exposed to cigarette smoke, the impaired synthesis or degradation of AnxA1 influenced immune responses through T helper cells [23]. Besides, the protective actions of Ac2-26 were demonstrated in bleomycin-induced pulmonary fibrosis [19] and pulmonary endotoxemic by local or systemic administration of lipopolysaccharide (LPS) [24]. In both models the mimetic peptide treatment reduced leukocyte influx and the release of proinflammatory cytokines, attenuating the inflammatory process. Recently, our research group showed the protective effects of the AnxA1 mimetic peptide Ac2-26 in an initial COPD model [25].

In view of the above, in this investigation, we performed a cigarette smoke cessation model to evaluate the AnxA1 profiles in the ongoing and post cigarette smoke exposure to reinforce the protein as a possible therapeutic alternative in the management of COPD and persistent airway inflammatory responses.

2. Material and methods

2.1. Animals and protocols of exposure to cigarette smoke

Wistar rats (200 g body weight) were divided into three groups (n = 6/ group) and kept in individual cages in a controlled environment (24–25 °C, 12 h light/dark cycle) with water and food *ad libitum*. All experimental procedures were conducted according to the guidelines for biomedical research stated by the Brazilian Societies of Experimental Biology and approved by the Ethics Committees on Animal Use at University Center Padre Albino (Certificate nº 05/14), Brazil. The experiments were designed to minimize the number of animals used and their suffering during the execution of the protocols. All animals were daily evaluated by the veterinarian of the institution.

After one week of acclimatization period, two groups of animals were exposed to cigarette smoke for 8 weeks in a specific smoke exposure apparatus [26,27]. The apparatus consists of an animal containment system and a cigarette smoke release system with an external cigarette holder connected to a dynamic suction pump. The pump can be programmed so that cigarette suction periods alternate with periods of clean air suction to prevent asphyxiation. Exposures were standardized and the animals exposed to the burning of 10 commercial cigarettes (containing 0.8 mg of nicotine, 10 mg of tar and 10 mg of carbon monoxide), one after the other, resulting in approximately 1 h of exposure, twice a day (total of 20 cigarettes/ day). The first exposure was performed in the morning (7 a.m.) and the second in the early evening (6 p.m.) [27].

On the day after the ending of the exposure protocol, animals from the chronic group (CS) were euthanized by excessive dose of anesthetic (thiopental). The smoking cessation group (SC) remained other 8 weeks without exposure to cigarette smoke [11,12]. The control group (C) was kept in the same conditions but only exposed to compressed air [27].

2.2. Quantitative analyses of bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was obtained at the end of the experiment. The animals had the trachea cannulated and the right lung clamped. The left lung was washed 3 times with 500 µl of PBS and the collected liquid was centrifuged for 10 min at 1500 rpm. The supernatant was stored at −70 °C for subsequent biochemical and cytokine assays. The pellet was resuspended in 500 µl PBS and 10 µl aliquots were stained in Turk (1:10) for quantification of inflammatory cells in a Neubauer camera (values as number of cells x 10^5 / ml).

2.3. Biochemical blood assays and plasma cytokines levels

Blood was collected by cardiac puncture in heparinized syringes, aliquots were centrifuged for 15 min at 3000 rpm and the plasma frozen at −70 °C for further biochemical evaluations and cytokine measurements. Glucose (Cat No: 133-1/500) and gamma-glutamyltransferase (gamma-GT) (Cat No: 105-2/30) were measured by means of commercial kits (LAB Test, Minas Gerais, Brazil) in a spectrophotometer (absorbance 540 nm).

IL-1β, IL-6 and IL-10, tumor necrosis factor (TNF)-α and the monocyte chemoattractant protein (MCP)-1 were quantified in blood plasma using the rat cytokine MILLIPLEX MAP Kit (RECYTMAG-65 K; Millipore Corporation, USA) according to manufacturer's instructions and analyzed on the LUMINEX xMAP MAGPIX equipment (Millipore Corporation, USA).

2.4. Histopathological studies and quantification of mast cells

Trachea and right lung were removed, fixed in 4% formaldehyde and processed for paraffin inclusion. Sections of 5 µm were used for histopathological, morphometric and immunohistochemical analyses in a Leica microscope (DM500). For histopathological studies, the tissue sections were stained with Hematoxylin and Eosin (HE). Morphometric studies were performed by means of pulmonary alveolar area measurements using an image analyzer (Software Leica Image Analyses).

The mast cells were stained with 0.1% Toluidine Blue and analyzed according to their intact or degranulated morphological characteristics. Quantification of mast cells was performed in 10 images per slide obtained by the 40x objective in the Leica microscope (DM500) and the tissue areas were obtained in the image analyzer [27,28].

2.5. Immunohistochemical studies

Immunohistochemical studies were used to evaluate the expressions of the anti-inflammatory protein AnxA1 and the nuclear factor (NF)-kB as well as to perform the macrophages quantification in lungs. Sections were processed for antigenic recovery with citrate buffer pH 6.0, blockade of the endogenous peroxidase activity and incubation with the rabbit polyclonal primary antibodies: anti-AnxA1 (1: 1000), anti- NF-kB (1: 200) and anti-ED-1 (1: 150) (Zymed Laboratories, Cambridge, UK) for 12 h. They were then incubated with the biotinylated secondary antibody (Histostain Kit, Invitrogen) and immersed in a conjugated streptavidin-peroxidase complex. The substrate diaminobenzidine (DAB Kit, Invitrogen) was used for the development and, thereafter, the

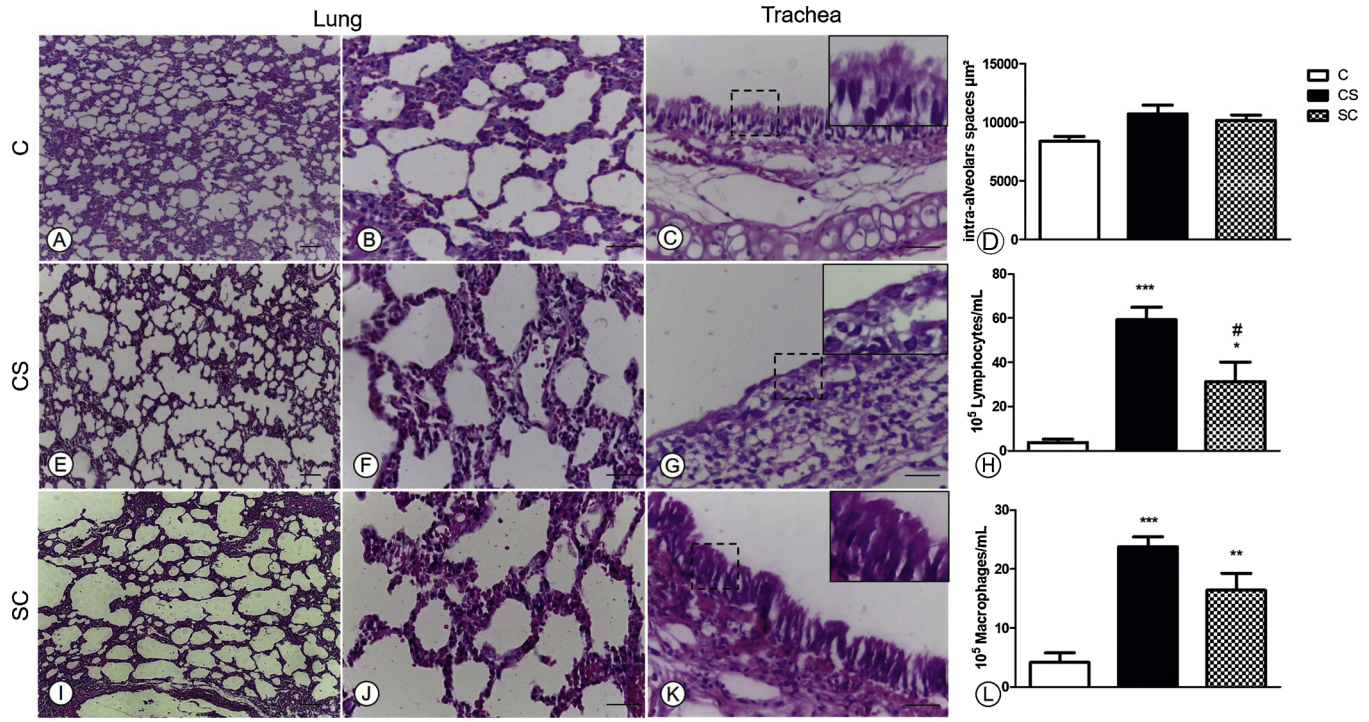

Fig. 1. Histopathological analysis. Lung - Control group (A and B), chronically-exposed-to-smoke (CS) (E and F) and smoking cessation (SC) animals (I and *J*) with increased intra-alveolar spaces and influx of inflammatory cells. Color: Hematoxylin-Eosin. Bars: 10 µm. Morphometry of intra-alveolar spaces (D) showed no significant difference among groups ($p = 0.17$). Results presented as mean ± S.E.M. ($N = 6$ / group). **Trachea** - Control (C), chronically-exposed-to-smoke (CS) with lining tissue alterations and loss of cilia (G) but recovery of cilia in smoking cessation group (CS) (K). Color: Hematoxylin-Eosin. Bars: 10 µm. **Quantitative analysis of bronchoalveolar lavage** - Quantification of lymphocytes (H) and macrophages (L) in Neubauer camera. Results presented as mean ± S.E.M. ($N = 6$ / group), * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ vs control; # $p < 0.05$ vs chronically-exposed-to-smoke.

sections were stained with Hematoxylin. Proteins were quantified by densitometry as arbitrary units from 0 to 255 using the Leica Image Analysis software image analyzer [27,28]. Macrophages were quantified as previously described for mast cells.

2.6. Statistical analyses

The results were submitted to descriptive analysis and determination of normality. Afterwards, the Analysis of Variance (ANOVA) was used followed by the Bonferroni test. AnxA1 was correlated to NF-kB by the Pearson test. All values were expressed as mean ± S.E.M. and P values less than 0.05 were considered statistically significant. The correlation results were indicated as positive or negative.

3. Results

3.1. Increased intra-alveolar spaces remain after smoking cessation but lining tissue recovery occurs in trachea

Histopathological studies of the lung revealed an influx of inflammatory cells in the chronic (CS) (Fig. 1E and F) and smoking cessation (SC) (Fig. 1I and J) groups. Enlargement of intra-alveolar spaces were observed in both groups exposed to cigarette smoke compared to control, but the morphometric analysis showed no statiscal significance ($p = 0.17$; CS: 10,723 ± 738.3 µm²; SC: 10,174 ± 431.7 µm²; C: 8367 ± 413.6 µm²) (Fig. 1A, B and D).

In trachea, histopathological studies showed the loss of cilia of the respiratory epithelium in the chronically exposed-to-smoke group (Fig. 1G). However, recovery of cilia was observed in SC animals (Fig. 1K).

Quantification of inflammatory cells in BAL (Fig. 1) indicated significant increase of lymphocytes (CS: $59.20 \times 10^5 \pm 5.791 \times 10^5$ lymphocytes/mL, $p < 0.001$; SC: $31.20 \times 10^5 \pm 8.952 \times 10^5$ lymphocytes/mL, $p < 0.05$) (Fig. 1H) and macrophages (CS: $23.80 \times 10^5 \pm 1.655 \times 10^5$ macrophages/mL, $p < 0.001$; SC: $16.40 \times 10^5 \pm 2.857 \times 10^5$ macrophages/mL, $p < 0.01$) (Fig. 1L) in the groups exposed to cigarette smoke in relation to control animals ($3.800 \times 10^5 \pm 1.463 \times 10^5$ lymphocytes/mL; $4.200 \times 10^5 \pm 1.594 \times 10^5$ macrophages/mL). Smoking cessation promoted reduction of lymphocytes compared to CS group ($p < 0.05$).

3.2. Smoking cessation partially reduced biochemical changes and levels of inflammatory mediators

The biochemical analysis indicated that glucose and gamma-GT levels were increased in chronically exposed-to-smoke animals (glucose: 415.5 ± 26.52 mg/dL; gamma GT: 19.83 ± 0.9474 U/L) ($p < 0.001$) compared to control (glucose: 129.5 ± 18.53 mg/dL; gamma-GT: 7.110 ± 1.030 U/L) (Fig. 2A and B) and reduced after smoking cessation (glucose: 167.3 ± 3.106 mg/dL, $p < 0.001$; gamma GT: 11.09 ± 2.142 U/L, $p < 0.01$).

Higher levels of IL-1β (303.2 ± 24.44 pg/mL, $p < 0,001$) (Fig. 2C), IL-6 (0.02195 ± 0.008393 pg/mL, $p < 0,05$) (Fig. 2D), IL-10 (0.0246 ± 0.002750 pg/mL, $p < 0,001$) (Fig. 2E), MCP-1 (2.597 ± 0.4057 pg/mL, $p < 0,001$) (Fig. 2F) and TNF-α (5.368 ± 0.6791 pg/mL, $p < 0,001$) (Fig. 2G) were observed in the blood plasma of chronic group compared to control (IL-1β: 9.210 ± 0.3597 pg/mL; IL-6: 0.0008833 ± 0.0003167 pg/mL; IL-10: 0.0021 ± 0.0003706 pg/mL; MCP-1: 0.6426 ± 0.06900 pg/mL; TNF-α: 0.2344 ± 0.04509 pg/mL). Smoking cessation promoted decreased levels of IL-1β (198.4 ± 13.86 pg/mL, $p < 0,001$), IL-6 (0.002691 ± 0.001632 pg/mL, $p < 0,05$) and MCP-1 (1.219 ± 0.2649 pg/mL, $p < 0,05$). Although non-significant lower levels of TNF-α (3.342 ± 0.7225 pg/mL) were found in SC group, increased IL-10 concentration (0.05738 ± 0.004741 pg/mL, $p < 0,001$) was observed by smoking cessation.

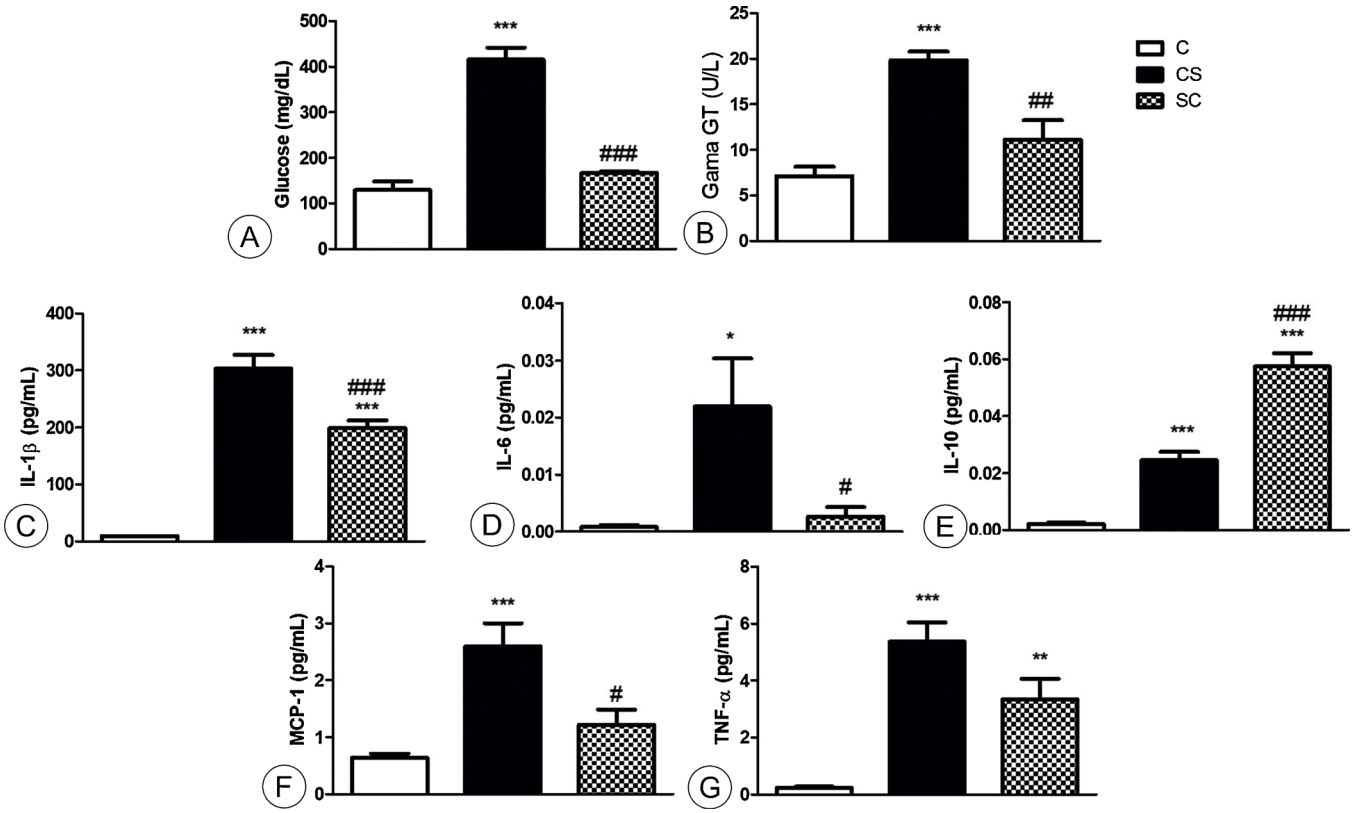

Fig. 2. Blood biochemical analyses. Glucose (A) and gamma GT (B) measurements were performed in the total blood, respectively by mg / dL and U / L. **Levels of cytokines in blood plasma** - IL-1β (C); IL-6 (D); IL-10 (E); MCP-1 (F); TNF-α (G). Results presented as mean ± S.E.M. (N = 6 / group). * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$ vs control; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ vs chronically-exposed-to-smoke. There was non-significant difference ($p > 005$) between SC and CS for TNF-α.

3.3. Number of mast cells and macrophages similar to control by smoking cessation

Large number of mast cells, mainly degranulated (Fig. 3B and H), and numerous macrophages in the intra-alveolar spaces and inter-alveolar septa (Fig. 3E and I) were observed in CS group (43.13 ± 0.2795 mast cells/mm^2; 91.50 ± 3.488 macrophages/mm^2) compared to control (15.42 ± 1.728 mast cells/mm^2; 13.67 ± 0.9189 macrophages/mm^2; $p < 0.001$) (Fig. 3A, D, H and I), however, smoking cessation reduced both cells (17.92 ± 1.845 mast cells/mm^2; 12.50 ± 3.651 macrophages/mm^2; $p < 0.001$) (Fig. 3C, F, H and I). The specificity of the immunolabeling was confirmed by the respective reaction controls (Fig. 3G).

3.4. Smoking cessation leads to overexpression of AnxA1 and reduction of NF-kB

The AnxA1 and NF-kB immunostainings were observed in the cytoplasm in all groups (Fig. 4A–F), while the NF-KB expression was also observed in the nucleus in the CS group (Fig. 4E).

Increased expressions of the AnxA1 (173.9 ± 6.802 arbitrary units; $p < 0.05$) (Fig. 4B and H) and NF-kB (188.4 ± 6.283 arbitrary units; $p < 0.001$) (Fig. 4E and I) were observed in the lungs of the chronically exposed-to-smoke group compared to normal tissue (AnxA1: 145.1 ± 4.692 arbitrary units; NF-kB: 149.8 ± 3.114 arbitrary units) (Fig. 4A, D, H and I). Interestingly, in SC group elevated expression of AnxA1 (177.3 ± 9.040 arbitrary units) (Fig. 4C and H) and reduction of NF-kB (163.1 ± 4.925 arbitrary units) ($p < 0.01$) (Fig. 4F and I) occurred. The specificity of the immunolabeling was confirmed by the respective reaction controls (Fig. 4G).

A positive correlation was found between AnxA1 and NF-kB in the chronically-exposed-to-smoke group (Table 1). In contrast, AnxA1 and NF-kB showed a negative correlation with smoking cessation.

4. Discussion

Exposure to cigarette smoke may result in chronic inflammation, such as COPD [29] and, consequently, there is interest in the search for anti-inflammatory treatments. Smoking cessation promotes partial reversal of the inflammatory process [8–12] and the understanding of the role of endogenous proteins with this anti-inflammatory profile, such as AnxA1, may be interesting for future interventions related to recovery from diseases caused by smoking [7]. In this research, we analyzed histopathological and biochemical characteristics, as well as the expression of the AnxA1 protein in a smoking cessation model.

Morphometric and histopathological studies of the lungs and bronchoalveolar lavage analyses showed that exposure to cigarette smoke promoted enlargement of intra-alveolar spaces, increased pulmonary congestion and leukocyte influx which reinforced previous data from our research group [27]. Even after eight weeks of smoking cessation, the enlargement of the intra-alveolar spaces and the presence of many inflammatory cells were still observed, whereas the lymphocytes were reduced in the bronchoalveolar lavage. This partial reduction of the inflammatory process was also verified by other researches. Such as the presence of increased intra-alveolar spaces that was observed in mice after four weeks of smoking cessation [12] and also, in humans, persistent airway inflammation and emphysema progression were found in former smokers after four years of smoking discontinuity [9].

Differently, our histopathological evaluations of the trachea revealed a more pronounced tissue recovery after cessation of smoking, with a reappearance of the cilia in the lining epithelium. An investigation with former smokers on smoking abstinence for more than

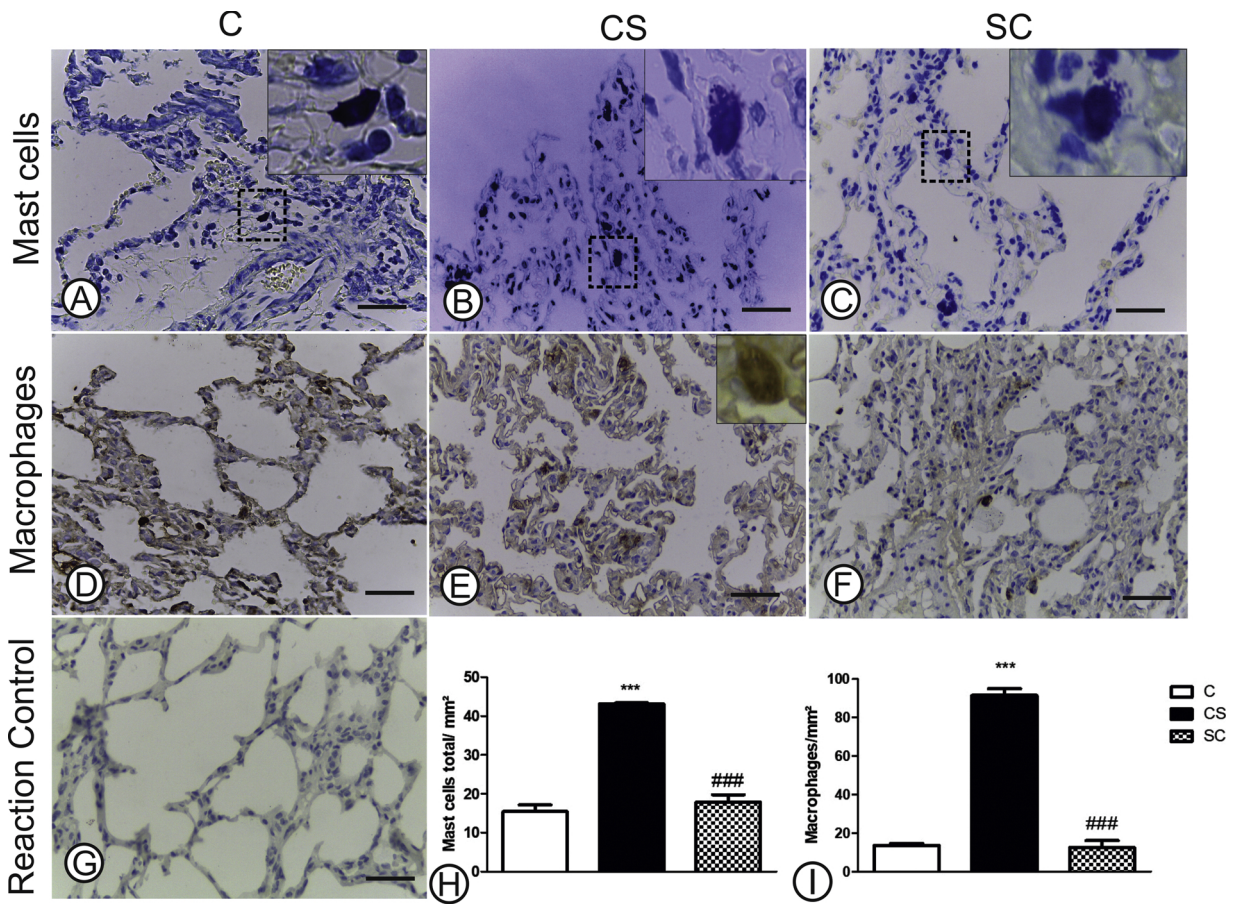

Fig. 3. Mast cells and macrophages in lung - Few mast cells (A) and macrophages (D) in control group. Numerous cells in the chronically-exposed-to-smoke group (B, E, H and I) and reduction after smoking cessation (C, F, H and I). (N = 6 / group). Staining: Toluidine blue (A, B and C). Counter-staining: Hematoxylin (D, E and F). Absence of immunostaining in the control of the reaction (G) Bars: 10 μm. Results presented as mean ± S.E.M. *** $p < 0.001$ vs control; ### $p < 0.001$ vs smoking cessation group.

one year showed normal bronchial epithelium only in former smokers without COPD, but progressive changes were observed in patients with a more advanced disease [10]. These authors associated the impaired mucosal immunity with persistent airway inflammation and progressive airway remodeling in COPD.

Our biochemical measurements of blood plasma indicated increased levels of glucose and gamma GT in chronically exposed-to-smoke rats, however, after smoking cessation, these levels were similar to controls. Biochemical alterations of glucose were observed in male and female offsprings exposed to tobacco smoke during lactation [30] and elevated levels of gamma-GT were also found in exposed-to-smoke rats in our previous studies [27]. In humans increased waist circumference, white blood cells, serum triglycerides, gamma-GT and C-reactive protein were observed in smokers [31]. In addition, higher levels of serum gamma-GT, fasting plasma glucose and insulin resistance index of homeostatic model assessment (HOMA-IR) were found in smokers with chronic kidney disease. [31].

Levels of inflammatory mediators were evaluated in blood plasma. As expected, elevated levels of IL-1β, IL-6, TNF-α and MCP-1 were detected in the chronically exposed-to-smoke group as a result of continuous lung inflammation [25,27,32,33]. However, in the smoking cessation group, these inflammatory mediators were reduced as observed by the administration of the AnxA1 mimetic peptide in an initial COPD model [25]. Interestingly, IL-10 showed increased levels, mainly after smoking cessation, indicating the body's own defense mechanism against the inflammatory process of the lung [34].

In the continuity of the research, we proceeded to the analysis of the inflammatory cells. Higher numbers of mast cells and macrophages were found in the chronically exposed-to-smoke group with a reduction after cessation of smoking. The higher number of neutrophils, macrophages and mast cells was associated with the involvement of these cells in the development of small airway dysfunction in smokers [35]. Mast cells altered the cellular environment and may have contribute to the progression of COPD [36]. These cells vary in number, phenotype and distribution besides, they increase in the lungs and small airways in COPD [37–39].

Also, activated macrophages secrete various mediators of inflammation, which, uncontrolled, can lead to tissue destruction and fibrosis, characteristics of COPD [40], while alveolar macrophages increase in an attempt to cleanse the toxins from cigarette smoke. Moreover, the increased release of chemical mediators by these cells occurs following exposure to cigarette smoke [8]. Furthermore, the expanded macrophage population was associated with COPD persistence in mice [11]. The reduction of macrophages observed by smoking cessation in our investigation may be associated with decreased MCP-1 levels also found in this study.

After checking the partial attenuation of the inflammatory process promoted by smoking cessation, we performed the immunohistochemical studies to verify the expression profiles of the AnxA1 and NF-kB proteins. Increased expressions of AnxA1 and NF-kB were observed in the chronically-exposed-to-smoke group. Interestingly, in smoking-abstinent-animals, elevated expression of AnxA1 occurred with concomitant reduction of NF-kB. In a model of gout in mice submitted to a high-fiber diet and short-chain fatty acid acetate treatment, the resolution of neutrophilic inflammation was associated with decreased NF-kB expression and enhanced IL-10 level and

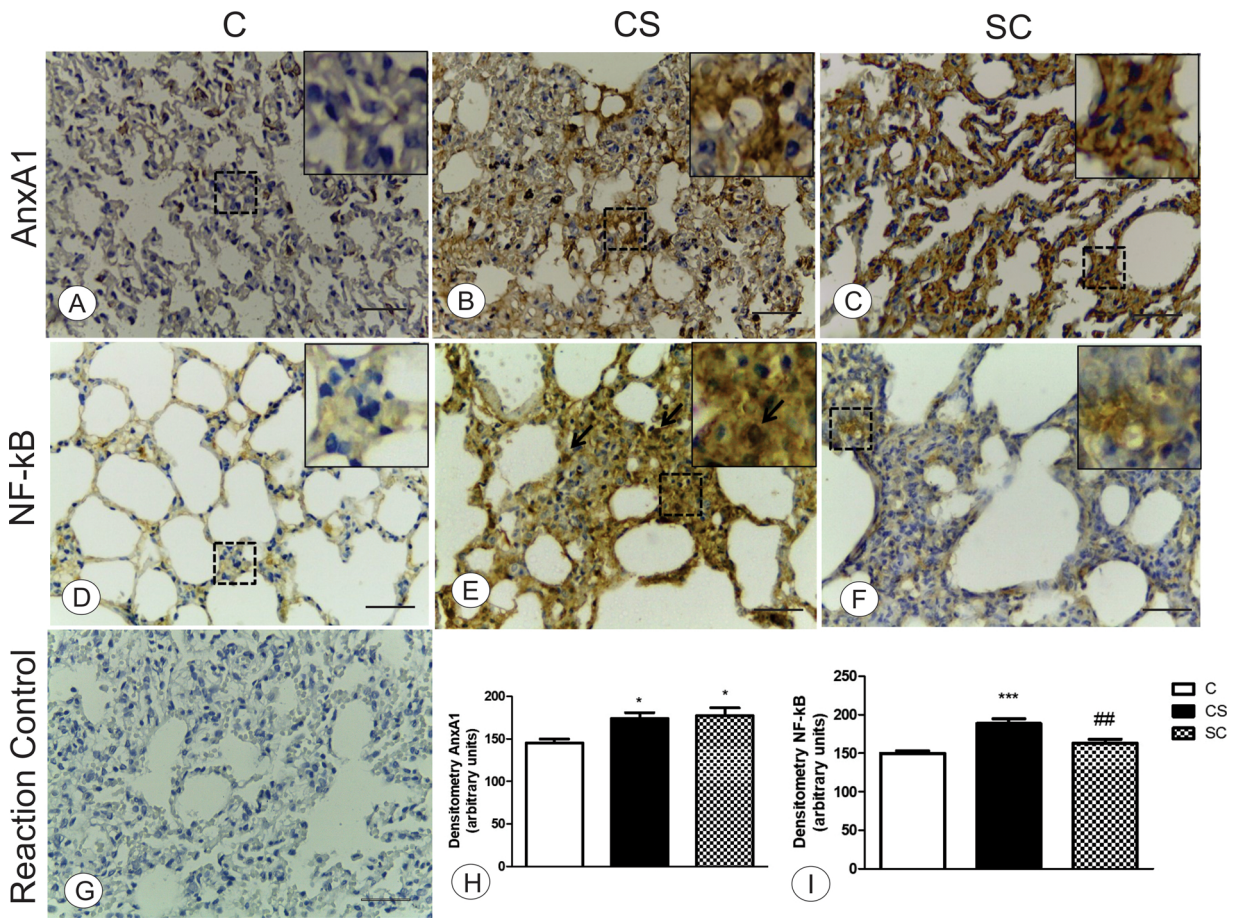

Fig. 4. Expression of the AnxA1 and NF-kB - Reduced expression of both proteins in the lung and trachea of the control group (A, D, H and I), increased immunolabeling in the chronically-exposed-to-smoke (CS) (B, E, H and I). Elevated erexpression of AnxA1 and reduction of NF-kB in the smoking cessation group (C, F, H and I). The insets show magnified areas of dashed box (A, B, C, D, E, F). Arrows indicate nuclear immunolabeling (E). Absence of immunostaining in the control of the reaction (G). Counterstaining: Hematoxylin. Bars: 10 μm. Results presented as mean ± S.E.M. (N = 6 / group). * $p < 0.05$; *** $p < 0.001$ vs control; ## $p < 0.01$ vs chronically-exposed-to-smoke.

Table 1
Correlation between ANXA1 and NF-kB expression levels in lung samples.

Anxa1	NF-kB
Control	149.8 ± 7.627 R = 0.6304 P = 0.1797
CS	190.2 ± 13.29 R = 0.8304 P = 0.0407*
SC	165.2 ± 13.01 R = - 0.8824 P = 0.0199*

R = Correlation coefficient; p value: Pearson's correlation; * significant difference.

AnxA1 expression [41]. Overexpression of the AnxA1 protein in PC12 cell culture (derived from phaeochromocytoma of the adrenal medulla) was also associated with decreased expression of NF-kB and IL-6 levels [42]. In addition, Ac2-26 administration in acute lung injury induced by ischemia reperfusion in rats attenuated lung edema, neutrophil infiltration, inflammatory cytokine release, oxidative stress, apoptosis and translocation of NF-kB [43]. The increase of AnxA1 was also observed in patients with severe COPD which was associated with fibrosis [21]. The failure of the protein to contain the inflammation may be related to the deregulated inflammatory process [21].

AnxA1 and other anti-inflammatory mediators converge to FPR2, an important receptor in COPD [7]. The contribution of AnxA1 and the FPR2 receptor has been investigated in the regulation of inflammatory responses in normal human lung fibroblasts [44]. The silencing of AnxA1 expression in fibroblasts by small interfering RNA (siRNA) and FPR2 blockade was linked to significant increases in TNF-α and IL-6, accompanied by decrease in the extracellular regulatory signal (ERK) [44]. The deletion of the AnxA1 gene in mice led to increased cyclooxygenase-2 (COX-2) and cytosolic phospholipase A2 (cPLA2), and an exaggerated response to inflammatory stimuli characterized by augmented leukocyte migration and IL-1β production [45]. The treatment of lung carcinoma cell lines with green tea (*Camellia sinensis*) induced increased expression of AnxA1 and reduced COX-2 expression [46]. These authors also showed that silencing of AnxA1 abolished the inhibitory activity of green tea on COX-2, indicating that this anti-inflammatory action is mediated, at least in part, by overexpression of AnxA1. Besides, the importance of the administration of AnxA1 in the reversal of inflammatory lung processes in animal models was demonstrated by the reduction of the influx of inflammatory cells in the lungs, as well as the decrease in the release of transforming growth factor (TGF-β1), interferon gamma (IFN- ϒ) and TNF-α [19], but increased IL-10 levels [24], suggesting that AnxA1 has therapeutic potential in chronic inflammatory lung diseases.

More recently our research group highlighted the protective role of the Ac2-26 mimetic peptide in initial COPD [25] by the attenuation of physiological and histopathological alterations, decreased inflammatory influx into the BAL, trachea and lungs. The treatment with Ac2-26 decreased hemoglobin, glucose, cholesterol, gamma glutamyl

transferase and aspartate aminotransferase blood levels. Similarly, reduction of proinflammatory mediators and higher concentration of anti-inflammatory cytokine were found in macerated lung supernatant, blood plasma and BAL in the treated animals. Reduced tissue expressions of AnxA1, COX-2 and metalloproteinase-9 were also observed [25]. The reduction in AnxA1 expression by administration of the exogenous peptide is probably associated with a negative feedback mechanism [47].

Because of the strong anti-inflammatory activity of AnxA1, we believe that the attenuation of the inflammatory influx into the BAL and lung tissues, as well as the reduction of anti-inflammatory mediators and NF-kB are associated with increased expression of endogenous AnxA1 that occurred in the chronically-exposed-to-smoke group, especially in the smoking cessation group.

5. Conclusions

Smoking cessation promoted partial recovery of morphological alterations in the lungs and trachea with reduction of tissue mast cells, macrophages and also proinflammatory chemical mediators in the blood plasma. This inflammatory process attenuation may be related to elevated erexpression of the AnxA1.

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