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Mimetic peptide AC2-26 of annexin A1 as a potential therapeutic agent to treat COPD

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is related to inflammatory process caused by smoking habit. In this scenario, the anti-inflammatory protein Annexin A1 (AnxA1) may represent a therapeutic alternative. We performed experiments to evaluate the effects of the AnxA1 mimetic peptide Ac2-26 in an initial COPD model by physiological, histopathological, biochemical and immunohistochemical analyses. Weight loss, increased blood pressure, reductions in the pulmonary frequency and ventilation, loss of tracheal cilia, enlargement of the pulmonary intra-alveolar spaces and lymphoid tissue found in untreated smoke-exposed group were attenuated by AnxA1 peptide treatment. The Ac2-26 administration also protected against leukocytes influx in bronchoalveolar lavage (BAL), lung and trachea, and it also led to decreased hemoglobin, glucose, cholesterol, gamma glutamyl transferase and aspartato aminotransferase 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. Besides Ac2-26 group showed reduced tissue expressions of AnxA1, cyclooxygenase-2 and metalloproteinase-9, but formylated peptide receptor 2 (FPR2) overexpression. Our results all together highlighted the protective role of the Ac2-26 mimetic peptide in COPD with promising perspectives.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a serious health condition estimated to be the third cause of death in 2020 [1,2]. The disease is characterized by progressive limitation of airflow and can be associated with the smoking habit [2,3]. Although COPD has a high incidence in men worldwide, the number of affected women has considerably increased at an alarming rate. In addition, women may respond to tobacco exposure with augmented oxidative stress, enhanced risk of airflow obstruction and impairment of lung function compared to men [4–7].

COPD is presented according to the progressive intensity of the smoking habit [8]. However, in animal models, it has been reported that the reduction in the time of exposure to tobacco associated with increasing of the cigarettes number per day produces similar results to those found by long-term exposure [9–11]. In a rat model of COPD [9], after 4 weeks of tobacco smoke exposure for 6 h a day and 3 days a week, signs of alveolar damage and the presence of neutrophils within the lung parenchyma were observed. The significant increases in airspace enlargement after 4 and 12 weeks of tobacco smoke exposure were similar, and an increase in total leukocytes recovered from bronchoalveolar lavage (BAL) was also showed after 4 weeks of tobacco

Abbreviations: μL, Microliter; μm, Micrometer; Ac2-26, Mimetic peptide Ac2-26 of annexin protein; AnxA1, Annexin A1 Protein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BAL, Bronchoalveolar lavage; BALT, Bronchus associated lymphoid tissue; COPD, Chronic obstructive pulmonary disease; COX-2, Cyclooxygenase 2; CS, Cigarette smoke; CS + Ac2-26, Cigarette smoke with mimetic peptide Ac2-26; ED-1, Antibody macrophage marker; FPR, Formylated peptide receptor; G, Gram; Gamma GT, Gamma Glutamyl Transferase; HE, Hematoxylin-Eosin; HRP, Horseradish peroxidase; IL-1β, Interleukin-1beta; IL-6, Interleukin-6; IL-10, Interleukin-10; I.P., Intraperitoneal; L, Liter; LDL, Low density lipoprotein; LPS, Lipopolysaccharide; MCP-1, Monocyte chemotactic protein-1; Mg, Milligram; mL, Mililiter; mm, Milimiter; MMP-9, Matrix metalloproteinase −9; NF-κB, nuclear factor κB; P, Value of P (significance of the statistical test); PBS, Phosphate buffered solution; Pg, Picogram; RPM, Rotation per minute; S.E.M, Standard error of mean = Standard error of mean; TNF-α, Tumor necrosis factor-alpha; U/L, Ultra/liter; Vs, Versus

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smoke exposure [9]. Other research using a rat-COPD model [10] indicated that daily cigarette smoke exposure for 2 h during 5 weeks promoted respiratory lesions similar to those observed in the 13 weeks exposure time. Reduced body weight and significant increases in neutrophil counts were also found after 5-weeks of exposure [10].

The inflammatory process induced by the inhalation of noxious particles and gases leads to pathological alterations, including mucosal hypersecretion, structural changes in the airways and loss of alveoli [2,3].

In the development of the disease, macrophages and respiratory tract epithelial cells activated by inhaled irritants release chemical mediators [2,12] that attract leukocytes and mast cells into the airways [13]. The selectin family adhesion molecules, as L-selectin on the neutrophil surface and E- and P-selectins on the endothelial cell surface are related to cigarette smoke-induced attachment of polymorphonuclear cells to endothelial cells [14].

In addition, airway dysfunction by smoking is associated with high expression of cytokines released especially by mast cells [15]. The number of inflammatory cells in bronchial biopsies and induced sputum can be correlated with disease seriousness as well as lung function and health condition declines. The inflammatory mediators also increase in disease exacerbation [3]. Tumor necrosis factor (TNF)-α and interleukin (IL)-1β are also important in neutrophil margination and attachment to endothelial cells by stimulating the production of adhesion molecules [14]. The increase in monocyte chemokine protein (MCP)-1 levels and a reduction in anti-inflammatory cytokines may also be involved in the inflammatory process in smokers with asthma or COPD [16,17]. Besides, studies have shown that levels of inflammation serum biomarkers, including matrix metalloproteinase (MMP)-9 are higher in COPD patients and linked to the degree of airflow obstruction and mortality [18].

The central role played by inflammation in COPD indicates that the development of novel anti-inflammatory therapies is critical, in particular to slow down disease progression and ameliorate the control of exacerbations [3,19]. Among the anti-inflammatory mediators, the endogenous protein Annexin A1 (AnxA1), the first characterized member of the annexin superfamily [20–22], may represent an alternative therapy for the treatment of COPD and other diseases caused by smoking. AnxA1 is a calcium-dependent binding protein of 37 kDa that also binds to membrane phospholipids and is involved in the inhibition of eicosanoids and cytosolic phospholipase A2 syntheses induced by glucocorticoid [23–25]. Structurally, annexins comprise two domains, a small N-terminal region, which varies in length and composition, and a highly conserved C-central domain. The N-terminal domain is unique to each member of the superfamily, it confers the specific activities and functions of annexins and contains sites for post-translational processes, such as phosphorylation, glycosylation, and proteolysis [20,25–27].

In the inactive cells, AnxA1 predominantly has intracellular location, being translocated, after activation, to the cell surface where it interacts with G protein coupled transmembrane receptors, the formylated peptides receptors (FPRs) [20,27]. Studies indicate that the FPR2 receptor is present in activated lung epithelial and inflammatory cells being particularly important for the COPD resolution [21].

After discovering that the biological activity of AnxA1 could be reproduced by the first amino acids of the N-terminal portion of the protein (peptide Ac2-26), it became common practice to use these molecules in experimental models of inflammation [27–31]. Intravital microscopy analysis of inflamed vessels in vivo pointed the site of action of AnxA1 in adherent leukocytes and the administration of exogenous AnxA1 or its derived peptide, Ac2-26, reduced adhesion and migration of leukocytes in the endothelium of post-capillary venules [26]. Moreover, leukocyte detachment appears to be mediated by the shedding of L-selectin on the surface of inflammatory cells [23–25].

AnxA1 protein is strongly expressed in alveolar macrophages and in human and animal airway epithelial cells [28]. The impact of AnxA1 as a mediator in the control of inflammatory and fibrotic phases was studied in a model of bleomycin-induced pulmonary fibrosis using knockout mice for AnxA1 [29]. The absence of the protein caused increased inflammation degree and fibrosis rates as well as higher transforming growth factor (TGF)-β1, interferon (IFN)-ɤ and TNF-α levels. However, treatment with the mimetic peptide of AnxA1 reduced the signs of inflammation and fibrosis [28]. This protective effect of Ac2-26 was also investigated in a model of pulmonary endotoxemic by local or systemic administration of lipopolysaccharide (LPS) [29]. Pretreatment with the peptide was able to reduce leukocyte influx and proinflammatory cytokines while it increased the anti-inflammatory mediator IL-1β to blood plasma. Other research also showed altered lung functions and exacerbated inflammatory and fibrotic responses on AnxA1 knockout mice exposed to silica [31]. Besides, it was suggested that he impaired synthesis or degradation of AnxA1 may influence immune responses in animals exposed to cigarette smoke through T helper cells [13].

In view of the above, we performed the administration of the AnxA1 mimetic peptide, Ac2-26, in a cigarette smoke model as a possible therapeutic alternative in the management of COPD.

2. Material and methods

2.1. Animals

Female Wistar rats (n = 30), 6 weeks old, were obtained from the Didactic and Experimental Research Unit of University Center Padre Albino, in Catanduva,-São Paulo, Brazil. The animals were divided into 3 groups (n = 10/group): control, exposed to compressed air only (control), exposed to smoke without treatment (CS) and exposed to smoke and treated with Ac2-26 peptide (CS + Ac2-26). The rats were kept in cages in a temperature controlled environment (22 to 25 °C) 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 Ethic Committee on Animal Use at University Center Padre Albino (Certificate n° 01/15). 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 institution's veterinarian.

2.2. Exposure to cigarette smoke and treatment with Ac2-26 mimetic peptide protocols

Two groups of animals were induced to initial COPD in a specific smoke exposure apparatus. The equipment 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 [32,33] (Supplementary Video 1). The exposures were standardized and performed twice a day using commercial cigarettes (containing 0.8 mg of nicotine, 10 mg of tar and 10 mg of carbon monoxide). The animals were exposed to the burning of 10 cigarettes in the morning (7 a.m) and 10 cigarettes in the early evening (6 p.m.) [11,33], each exposure lasted approximately 1 h. The total exposure period was 5 uninterrupted weeks (35 days).

The anti-inflammatory efficacy of Ac2-26 the AnxA1 mimetic peptide (Ac-AMVSEFLKQAWFIENEEQEYVQTVK, Thermo Fisher Scientific, Grand Island, NY, USA) in protecting against inflammatory processes caused by exposure to tobacco smoke was evaluated in one of the smoke-exposed groups (n = 10) by the intraperitoneal (ip) administration of Ac2-26 at the dosage of 1 mg/kg in 100 μL of phosphate buffered (PBS) solution [28–33], once a day and before the first exposure to cigarette smoke. The treatment protocol started along with the protocol of exposure to cigarette smoke and had the same duration of 5 weeks.

The control group (n = 10) was kept in the same conditions but