

Aerosolized Polymerized Type I Collagen Reduces Airway Inflammation and Remodelling in a Guinea Pig Model of Allergic Asthma

Paola Moreno-Alvarez · Edgar Sánchez-Guerrero ·
Erasmó Martínez-Cordero · Rogelio Hernández-Pando ·
María G. Campos · Lucely Cetina · Blanca Bazán-Perkins

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Abstract Collagen-polyvinylpyrrolidone (Collagen-PVP) has been demonstrated to elicit immunomodulatory properties in different chronic inflammatory diseases. Nevertheless, its effects on asthma are still unknown. We have evaluated whether collagen-PVP could modulate airway inflammation and remodelling in a guinea pig model of allergic asthma. Sensitized guinea pigs were challenged with the allergen (ovalbumin) six times (at 10-day

intervals). From the third challenge on, animals were treated every 5 days with saline aerosols containing 0.16, 0.33, or 0.66 mg/ml of collagen-PVP ($n = 5$, respectively). Some guinea pigs, sensitized and challenged with saline as well as treated with 0 or 0.66 mg/ml collagen-PVP, were included in the study as control ($n = 7$) and sham groups ($n = 5$), respectively. From the first challenge on, ovalbumin induced a transient airway obstruction, measured by barometric plethysmography, which was not modified by collagen-PVP treatments. After the last allergen challenge, guinea pigs were anesthetized to obtain bronchoalveolar lavage (BAL) and the left lung caudal lobe. As expected, BAL cell count from allergen-challenged guinea pigs showed abundant neutrophils and eosinophils, as well as numerous tumor necrosis factor (TNF)- α -expressing granulocytes and macrophages in airway wall (determined by immunohistochemical assay). Neutrophilia and TNF- α -expressing leukocytes, from collagen-PVP treated animals, diminished from 0.16 mg/ml, and eosinophilia from 0.66 mg/ml of collagen-PVP doses. Histological changes induced by allergen challenges include thickening of connective tissue below airway epithelium and vascular wall widening of airway adjacent vessels; these changes were reduced by collagen-PVP treatment. Collagen-PVP seems to have anti-inflammatory and antifibrotic properties in this guinea pig asthma model.

P. Moreno-Alvarez
Departamento de Biología Celular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Insurgentes Sur 3700, CP 04510 Mexico, DF, Mexico

E. Sánchez-Guerrero · B. Bazán-Perkins (✉)
Departamento de Hiperreactividad Bronquial, Instituto Nacional de Enfermedades Respiratorias, Ismael Cosío Villegas, Calzada de Tlalpan 4502, Colonia Sección XVI, CP 14080 Mexico, DF, Mexico
e-mail: perkins@unam.mx

E. Martínez-Cordero
Departamento de Enfermedades Crónico-Degenerativas, Instituto Nacional de Enfermedades Respiratorias, Ismael Cosío Villegas, Calzada de Tlalpan 4502, CP 14080 Mexico, DF, Mexico

R. Hernández-Pando
Departamento de Patología Experimental, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, CP 14000 Mexico, DF, Mexico

M. G. Campos
Unidad de Investigación Médica en Farmacología, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico, DF, Mexico

L. Cetina
Division de Investigación Clínica, Instituto Nacional de Cancerología, San Fernando 22, CP 14080 Mexico, DF, Mexico

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Introduction

Asthma is an airway disorder that involves many pathogenic events. Asthma onset is generally asymptomatic and

occurs when a pathogen agent affects the airways of a susceptible individual, producing inflammatory processes that, by an unknown mechanism, modify airway responsiveness. Some stimuli, physical or chemical, can trigger episodic crises characterized by airflow obstruction and respiratory symptoms [1–3]. An intense inflammatory response occurs during asthma exacerbations, including a transient influx of neutrophils followed by eosinophil predominance [2, 4]. Eosinophils and neutrophils can induce airway injury by causing structural changes; this is known as airway remodelling [2, 5]. A hallmark of airway remodelling in asthma is the subepithelial mucosa enrichment of extracellular matrix components [6, 7]. Additionally, eosinophils and neutrophils contribute to inflammation persistence by releasing many proinflammatory mediators, such as tumor necrosis factor (TNF)- α . The expression of TNF- α is linked to asthmatic complications pointing out TNF- α modulation as a therapeutic target [8]. Despite the significant advances in asthma therapy, its prevalence and mortality have increased worldwide [9].

Collagen-polyvinylpyrrolidone (collagen-PVP) is a biodrug, made with a γ -irradiated mixture of atelopeptidic porcine type I dermal collagen and PVP, with anti-inflammatory and fibrolytic properties [10, 11]. In chronic skin and joint inflammatory processes, Furuzawa-Carballada and colleagues [11–13] have demonstrated that collagen-PVP down-regulates interleukin (IL)-1 β , IL-8, TGF- β , and TNF- α , which are key mediators of inflammatory responses. Moreover, collagen-PVP reduces the inflammatory lymphocytic infiltration and the degree of fibrosis and tracheal stenosis in dog tracheoplasty [14]. Likewise, collagen-PVP has been demonstrated to display antifibrotic and antioxidant properties in chronic cyclosporine nephrotoxicity in rat [15]. In humans, collagen-PVP has been proved to be safe, well-tolerated, and with no adverse events, in short and long administration periods [16]. To our knowledge, the effect of aerosolized collagen-PVP in airway inflammation has not been evaluated so far. This study was designed to determine the effect of aerosolized collagen-PVP on airways inflammation and remodelling in a guinea pig model of allergic asthma.

Materials and Methods

Healthy male guinea pigs (350–400 g), from Harlan Mexico (strain HsdPoc:DH) and bred at our institutional laboratory animal facilities, were used. Animals were maintained in filtered air-conditioned, 12/12-hr light/dark cycles, at $21 \pm 1^\circ\text{C}$ and 50–70% humidity, and fed with sterilized pellets (2040 Harlan Teklad Guinea Pig Diet) and

water ad libitum. The protocol was revised and approved by the Scientific and Bioethics Committees of the Instituto Nacional de Enfermedades Respiratorias in Mexico City.

Study Design

After an initial sensitization protocol, guinea pigs were intermittently exposed to the aerosolized allergen (ovalbumin, OVA) for up to 65 days, receiving a total of six allergen challenges. Separate groups of animals were treated with 0, 0.16, 0.33, or 0.66 mg/ml of collagen-PVP aerosols administrated every 5 days from day 35 to day 60. One hour after the last OVA-challenge, on day 65, guinea pigs were anesthetized and euthanized to obtain bronchoalveolar lavage (BAL) for cell count. Afterwards, lung samples were obtained to analyze both the changes in the amount of collagen stain in subepithelial mucosa by Masson trichrome and the extent of airway smooth muscle layer in airways and arterioles by light microscopy. Airway subepithelial mucosa was defined as the area between the basal edge of epithelial cells and the beginning of airway smooth muscle, a region containing mainly basement membrane and lamina propria. Finally, TNF- α expression in airway leukocytes was analyzed with immunohistochemistry.

Sensitization Procedure

Guinea pig antigen sensitization was performed as described previously [5]. Briefly, on the first day each guinea pig received a single intraperitoneal injection of OVA (0.06 mg/ml) with aluminum hydroxide (1 mg/ml) dispersed in saline solution. Sensitization was reinforced 8 days later with OVA aerosols (3 mg/ml saline) delivered for 5 min. Aerosols were produced by an US-1 Bennett nebulizer (flow, 2 ml/min), releasing mixed particles, 44% $<4\ \mu\text{m}$ in size, 38% of 4–10 μm , and 18% $>10\ \mu\text{m}$ (multistage liquid impinger, Burkard Manufacturing Co., Rickmansworth, Hertfordshire, UK). From day 15 onwards, guinea pigs were challenged every 10 days with OVA aerosols for 1 min (1 mg/ml in the first challenge, and 0.5 mg/ml in the subsequent five challenges). Guinea pigs that received inhalatory challenges with vehicle (saline solution) comprised the control group. All challenges were performed while the animal stayed in a barometric plethysmograph. Acute airway obstruction-induced by the antigenic challenge was recorded immediately after finishing OVA delivery, as described in the following section. This guinea pig model of allergic asthma lacks a significant late airway response and induces production of OVA-specific IgG₁ (data not shown).

Barometric Plethysmography

Whole-body single-chamber plethysmography for freely moving animals (Buxco Electronics Inc., Troy, NY, USA) was used to evaluate the pulmonary function indirectly according to principles previously described [17, 18]. Briefly, pressure inside the plethysmograph was measured through a differential transducer connected to a preamplifier. Because air in lungs is heated and humidified, during the inspiratory phase the volume of air inside the thorax is larger than the volume of air drawn by the animal from the plethysmographic chamber. This larger volume of air inside the thorax produces an increase in the plethysmographic chamber pressure and a decrease during the expiratory phase. The signal from the chamber was processed with computer-installed software (Buxco Biosystem XA v1.1) to calculate several respiratory parameters, including Penh (enhanced pause). This index was obtained by the formula [18]:

$$\text{Penh} = ((\text{Te} - \text{Rt})/\text{Rt})(\text{PEP}/\text{PIP})$$

[Te = expiratory time (s), Rt = relaxation time (s), PEP = peak expiratory pressure (cmH₂O), and PIP = peak inspiratory pressure (cmH₂O)]. The software was adjusted to include only breaths whose tidal volume is 1 ml or larger, minimal inspiratory time is 0.15 s, maximal inspiratory time is 3 s, and maximal difference between inspiratory and expiratory volumes is 10%. Five minutes after placing the guinea pig inside the plethysmographic chamber, a 5-min baseline Penh recording was initiated. One minute after OVA or vehicle administration, Penh was recorded at 5 and 10 min, and every 15 min thereafter. Because Penh was calculated for each breath, the software was adjusted to obtain both mean values from all breaths occurring during 15 s, and mean values over the last 5 min of each period.

Collagen-PVP Treatments

Some OVA-challenged guinea pigs received 0, 0.166, 0.33 or 0.66 mg/ml collagen-PVP dispersed in 20 ml of saline for 5 min ($n = 5-7$). Collagen-PVP doses and frequency of administration were selected in accordance to previous studies using collagen-PVP to treat inflammatory processes in situ [11, 12, 16]. Collagen-PVP aerosols were administered with an US-1 Bennett nebulizer at a flow rate of 2 ml/min every 5 days from day 35. Collagen-PVP was administered 1 h after OVA when it coincided with the OVA-challenge day. Five guinea pigs from the control group received 0.66 mg/ml of collagen-PVP to constitute the sham group.

BAL Cell Counts

One hour after the sixth OVA challenge, and when at least 50% of baseline Penh was reached [19], animals were

ethanized by an overdose of sodium pentobarbital (65 mg/kg) and then the trachea was cannulated. Using a syringe, 5 ml of saline solution (37°C) were introduced through the tracheal tube and gently recovered 1 min later. This procedure was repeated and the recovered fluid was mixed with the first BAL fluid. Total recovered fluid was immediately centrifuged for 10 min at 500×*g* (4°C); the cell pellet was resuspended in 1 ml of saline solution, and total cells were stained with Kimura stain and counted in a Neubauer hemocytometer. Volume was adjusted to obtain 1 × 10⁶ cells/ml and 50 µl of this mixture was deposited on a slide stainer (7120 Aerospray, Wescor Inc., Logan, Utah, USA) to be stained with Romanowsky stain for differential cell counting. Cell counts were expressed as number of cells per milliliter of BAL fluid. Cell viability >80% was confirmed by Trypan blue exclusion technique in each BAL. All smears were coded, and cells were counted in a blinded fashion.

Conventional Histology and Automated Morphometry Analysis

The left caudal lung lobe was dissected and fixed by manual perfusion of 10% neutral buffered formaldehyde solution via an intra-arterial route until lung lobe was exsanguinated. Lung fragments obtained by sagittal cutting were embedded in paraffin, and 4-µm-thick lung sections were stained with Masson trichrome stain.

Surface areas (µm²) of airway subepithelial mucosa, smooth muscle, and adjacent vessels vascular smooth muscle were determined by means of automated morphometry (Qwin, Leica Microsystems Imaging Solutions, Cambridge, UK). All measurements were conducted in six bronchi, six bronchioles, and six arterioles (~ 100 µm diameter) chosen randomly from each animal. Data were adjusted by length of the corresponding basement membrane and averaged to obtain the final result. Bronchus and bronchiole were identified by the presence or absence of cartilage in airway wall, respectively.

Immunohistochemistry

The same paraffin-embedded lung tissue blocks used for the morphometric study were used for immunohistochemistry. Sections (3 µm) were deparaffinized (55°C, 30 min) and rehydrated through submersion in graded alcohols (xylene, 1:1 xylene-alcohol, alcohol, and 70% alcohol for 10 min each, and finally rinsed in distilled water). Antigen retrieval was performed with 10 mM of citrate buffer, pH 6, for 5 min in a microwave oven. Samples were treated with hydrogen peroxide (3%) to quench endogenous peroxidase, and nonspecific sites were blocked later with horse serum (2%). Sections were incubated at 4°C overnight with

antibody to TNF- α (1:400, epitope corresponding to amino acids 77–233 representing mature TNF- α of human origin, rabbit polyclonal IgG; Santa Cruz). To detect the specific binding of this primary antibody, an R.T.U. Vectastain Universal Quick Kit was used (Vector Laboratories, Inc., Burlingame, CA, USA) in which tissues were incubated sequentially with blocking serum, a pan-specific secondary antibody, and streptavidin/peroxidase complex. Finally, 3-amino-9-ethyl-carbazole (BioGenex, San Ramon, CA, USA) was used as chromogen. Sections were counter-stained with Mayers hematoxylin. Slides were rinsed twice with 0.1% Tween 20 phosphate-buffered saline during the whole process. Sections from the same lung were processed without the primary antibody as control for non-specific binding of the secondary antibody. No positive staining was observed in nonspecific binding controls. Rabbit IgG (Southernbiotech, Birmingham, AL, USA) isotype control was negative. The percentage of TNF- α -positive cells in airway wall was obtained after counting positive and negative airway wall granulocytes and macrophages in six random fields at 200 $\times$ magnification.

Materials

Collagen-PVP (Fibroquel) is an endotoxin-free biocompound and was purchased from Aspid SA de CV, México City, México. Ovalbumin (chicken egg albumin) grade II and all stains for microscopy were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aluminum hydroxide was purchased from J.T. Baker (Phillipsburg, NJ, USA). Pentobarbital sodium was acquired from Pfizer, Toluca, Mexico.

Statistical Analysis

One- or two-way ANOVA followed by Dunnett's, Tukey's, or Bonferroni's tests were used in multiple comparisons. Statistical significance was set at two-tailed $P < 0.05$. Data

in the text and figures are expressed as mean $\pm$ standard error.

Results

OVA challenges induced a transient (30 min) airway obstruction. Penh basal values did not change in any group throughout the study. The maximal airway obstruction-induced by OVA-challenge was not modified by collagen-PVP treatments (Table 1). Saline challenges in control and sham groups did not induce airway obstruction.

Total and Differential Cell Counts in Collagen-PVP-Treated Guinea Pigs

Total cell and macrophage numbers in BAL were similar among groups. Total and differential cell counts in saline-challenged guinea pigs were similar between control and sham groups (Fig. 1).

Neutrophil, eosinophil, and lymphocyte counts increased significantly in OVA-challenged guinea pigs ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively; Fig 1). All collagen-PVP doses reduced significantly neutrophil counts ($P < 0.01$); also, 0.66-mg/ml collagen-PVP dose significantly decreased eosinophil counts ($P < 0.05$). Total cell, macrophage, and lymphocyte counts in OVA-challenge guinea pigs were not modified by collagen-PVP treatments (Fig. 1).

Effect of Collagen-PVP on Airway Structure

Subepithelial areas in bronchiole and bronchi from saline-challenged guinea pigs were similar between collagen-PVP- and saline-treated animals (Fig. 2). Allergen challenge induced a statistically significant increase of subepithelial mucosa area in bronchioles ($P < 0.01$) and bronchi ($P < 0.01$; Fig. 2d). Collagen-PVP treatment significantly

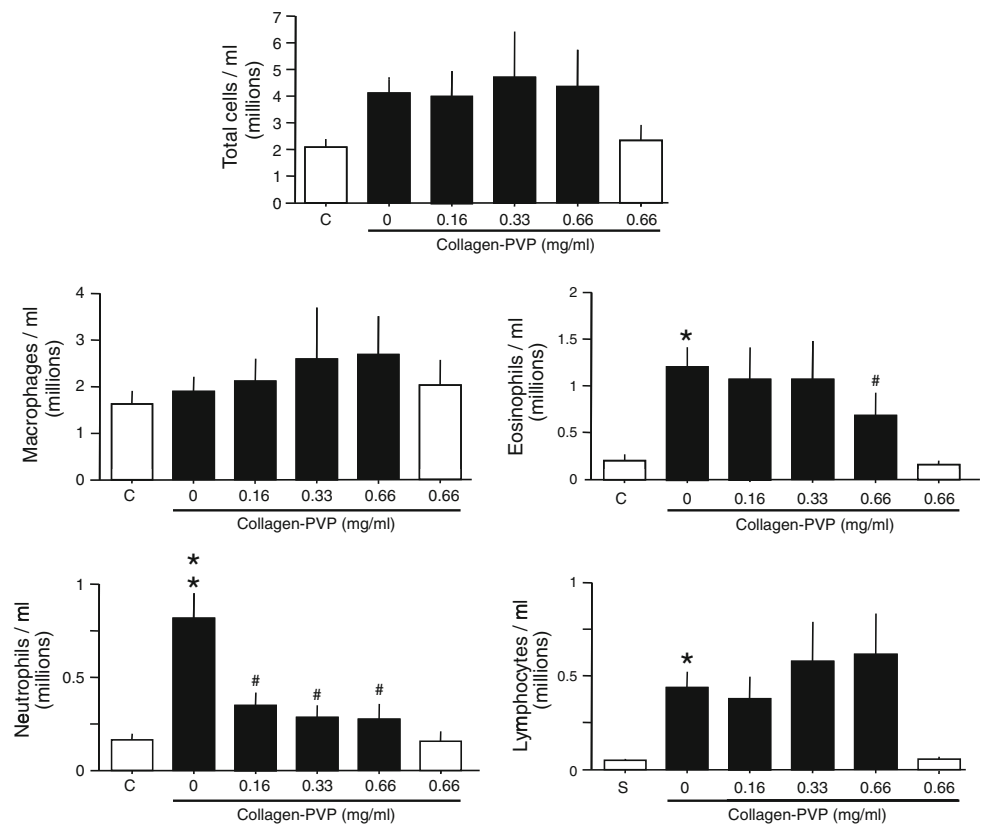
Table 1 Effect of collagen-PVP in maximum obstructive responses induced by allergen challenges

Groups	Challenge day				
	Second	Third	Fourth	Fifth	Sixth
0 mg/ml collagen-PVP (n = 7)	681.3 $\pm$ 198.2	745.2 $\pm$ 143.4	498.6 $\pm$ 68.9	487.7 $\pm$ 83.4	398.6 $\pm$ 96.5
0.166 mg/ml collagen-PVP (n = 5)	455.6 $\pm$ 65.5	742.0 $\pm$ 198.3	477.7 $\pm$ 68.5	445.3 $\pm$ 67.4	424.8 $\pm$ 65.6
0.33 mg/ml collagen-PVP (n = 5)	590.6 $\pm$ 217.4	594.3 $\pm$ 108.6	213.1 $\pm$ 48.1	607.4 $\pm$ 18.9	364.2 $\pm$ 57.5
0.66 mg/ml collagen-PVP (n = 5)	402.4 $\pm$ 91.2	518.9 $\pm$ 117.9	314.9 $\pm$ 79.1	553.5 $\pm$ 173.6	428.4 $\pm$ 27.2

The Penh response elicited by the antigenic challenge was devoid of statistical significance throughout the study (two-way ANOVA, Bonferroni posttest)

Data correspond to average values from maximum airway obstructive responses induced by allergen challenges obtained from second to sixth allergen challenges and measured as percentages of basal Penh acquired before allergen challenge

Fig. 1 Effect of collagen-PVP on the infiltration of inflammatory cells in guinea pig allergic airways. Cells recovered in BAL fluid are shown for each group. Bars and vertical lines are the mean $\pm$ standard error of the mean for control and sham groups ($n = 7$ and 5 , respectively, white bars). Black bars represent ovalbumin-challenged groups ($n = 5$ all groups). * $P < 0.05$ and ** $P < 0.01$ compared with control group (one-way ANOVA and Dunnett's multiple comparisons test). # $P < 0.05$ compared with 0-mg/ml collagen-PVP ovalbumin-challenged group (one-way ANOVA and Tukey's multiple comparisons test). C control group



reduced subepithelial mucosa area in bronchioles and bronchi: effect of 0.16 mg/ml dose was significant at $P < 0.05$, and 0.33- and 0.66-mg/ml doses at $P < 0.01$ (Fig. 2d). Airway smooth muscle areas were similar in all groups for bronchioles and bronchi (data not shown).

The arteriole wall area in control and sham groups was similar (Fig. 3). In contrast, the arteriole wall area in OVA-challenged guinea pigs was significantly larger ($P < 0.01$). The administration of aerosolized collagen-PVP reverted the widening of arteriole wall induced by OVA-challenges (Fig. 3D).

TNF- α Expression in Granulocytes and Macrophages in Collagen-PVP-Treated Guinea Pigs

The percentage of TNF- α immunoreactive granulocytes and macrophages in the airway wall of control and sham groups was similar (Fig. 4). In contrast, the analysis of lung tissue from OVA-challenged guinea pigs showed an increase of TNF- α immunoreactive granulocytes ($P < 0.01$) and macrophages ($P < 0.01$; Fig. 4). The treatment with 0.33- and 0.66-mg/ml collagen-PVP significantly reduced the TNF- α immunoreactivity of granulocytes ($P < 0.05$ and $P < 0.01$, respectively; Fig. 4), whereas each dose of collagen-PVP reduced the TNF- α immunoreactivity of macrophages

($P < 0.05$, 0.16 mg/ml collagen-PVP, and $P < 0.01$, 0.33, and 0.66 mg/ml collagen-PVP; Fig. 4).

Discussion

Although highly effective drugs are currently used for asthma control, none are useful to treat all asthma clinical phenotypes [20, 21]. Thus, the development of new anti-asthmatic therapies is always encouraged. Guinea pigs are susceptible to generate pulmonary hypersensitivity to several antigens and, as asthma model, are useful for testing novel therapeutics tools [22]. Collagen-PVP is a novel drug that has shown immunomodulatory, fibrolytic, hemostatic, and tissue regeneration properties [10–15, 22–24]. In this study, collagen-PVP reduced neutrophil infiltration, leukocyte TNF- α expression, and collagen deposition in a dose-independent manner in airway inflammation induced by repetitive allergen challenge.

Although asthma is a heterogeneous airway disorder, inflammation is persistently involved in its pathogenesis. Two predominant inflammatory asthma phenotypes have been described in humans: eosinophilic and neutrophilic [21]. Traditionally, eosinophils have been considered a key component of asthma and are the predominant inflammatory cell in mild-to-severe asthma. By releasing

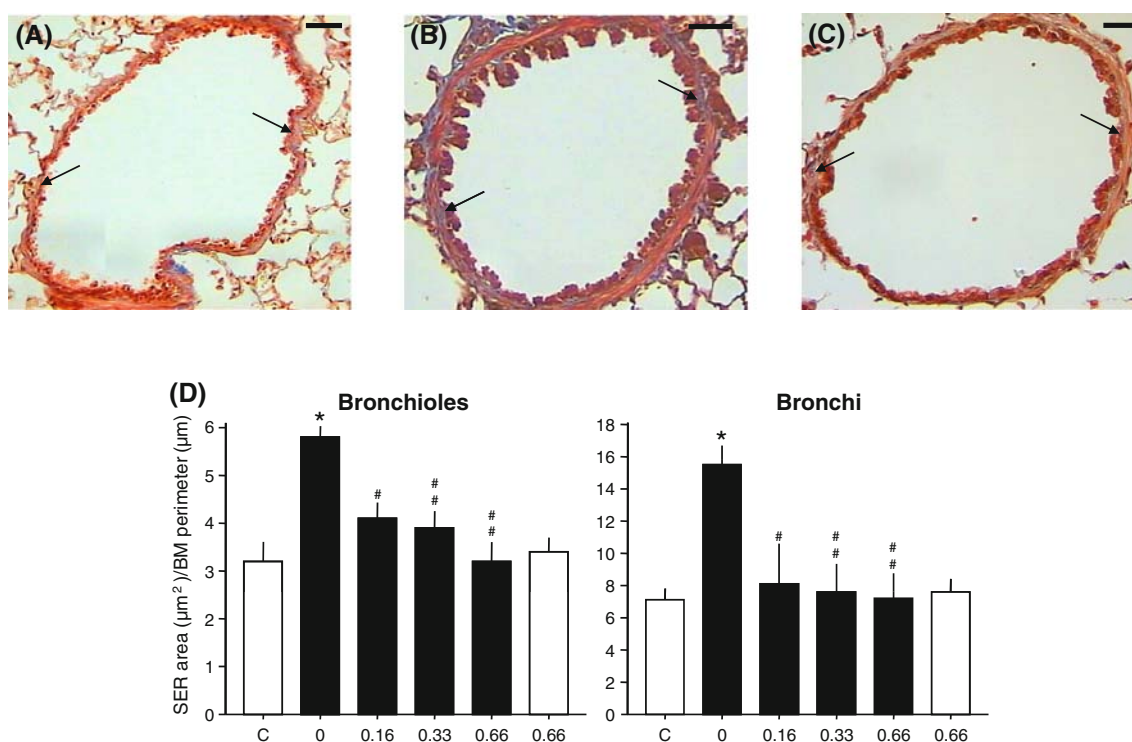


Fig. 2 Changes induced by collagen-PVP in subepithelial mucosa area in guinea pig allergic airways. **a** Micrograph from lung of control guinea pig showing a bronchiole. Note the small amount of fibrous tissue in the adventitial layer (arrows). **b** Lung from a sensitized guinea pig after the sixth OVA challenge. There is a slight increase of fibrous tissue (arrows) in the subepithelial layer and evident epithelial hyperplasia. **c** Lung section from a sensitized guinea pig after the sixth OVA challenge and treated with collagen-PVP (0.66 mg/ml). There is small amount of fibrous tissue around the bronchiole (arrows). Scale bars correspond to 50 μm. **d** Panels depict the areas of

subepithelial mucosa (standard error of the mean (SEM)), bronchi and bronchiole, adjusted by the basement membrane (BM) perimeter, as measured by automated morphometry. Bars and vertical lines are the mean ± SEM for control and sham groups ($n = 7$ and 5 , respectively, white bars). Black bars represent ovalbumin challenged groups ($n = 5$ all groups). * $P < 0.01$ compared with control group (one-way ANOVA and Dunnett's multiple comparisons test). # $P < 0.05$, ## $P < 0.01$ compared with 0-mg/ml collagen-PVP ovalbumin-challenged group (one-way ANOVA and Tukey's multiple comparisons test). C control group

proinflammatory cytokines, chemokines, lipid mediators, and toxic granule proteins, eosinophils contribute importantly to asthma pathophysiology, mainly in airway remodelling [25]. Nevertheless, it has been described that not all patients with asthma have airway eosinophilia. In addition, this noneosinophilic asthma represents a distinct clinical and pathological phenotype that needs to be treated differently [26]. As for neutrophils, they are common during acute exacerbations but also are implicated in the inflammation persistency observed particularly in severe and fatal asthma, in both acute and chronic settings [27]. Activated neutrophils are able to release mediators, such as leukotrienes, cytokines, chemokines, proteases, and reactive oxygen intermediates, capable to promote and prolong tissue injury during asthma [28]. We observed that repetitive allergen challenge in sensitized guinea pigs induced an immediate increase of eosinophil and neutrophil infiltration. Eosinophilic infiltration induced by allergen challenges was diminished only by 0.66-mg/ml collagen-PVP dose, whereas neutrophil infiltration was down-regulated in

a dose-independent manner, dose-independently by collagen-PVP. Neutrophilic inflammation is commonly resistant to glucocorticoids treatment, the first-line and long-term antiinflammatory therapy [20, 29]. Corticosteroids prolong neutrophil survival by inhibiting their apoptosis [20]. Although the precise effect of collagen-PVP on neutrophil infiltration control is unknown, it is noteworthy that this biodrug is a good modulator of neutrophil infiltration.

An interesting effect of collagen-PVP was the reduction of TNF- α expression in airway granulocytes and macrophages, because the last is a main source of TNF- α [30]. TNF- α has an important role in the normal response to infection and usually is not detectable in healthy individuals. Nevertheless, during a pathological process, such as chronic inflammation, TNF- α production increases and is excessive in severe asthma. TNF- α has a central role in asthma by promoting inflammation, including neutrophil recruitment, remodelling, and hyperresponsiveness. Recently, although a promissory anti-TNF- α therapy has been developed, it has been observed that only a small

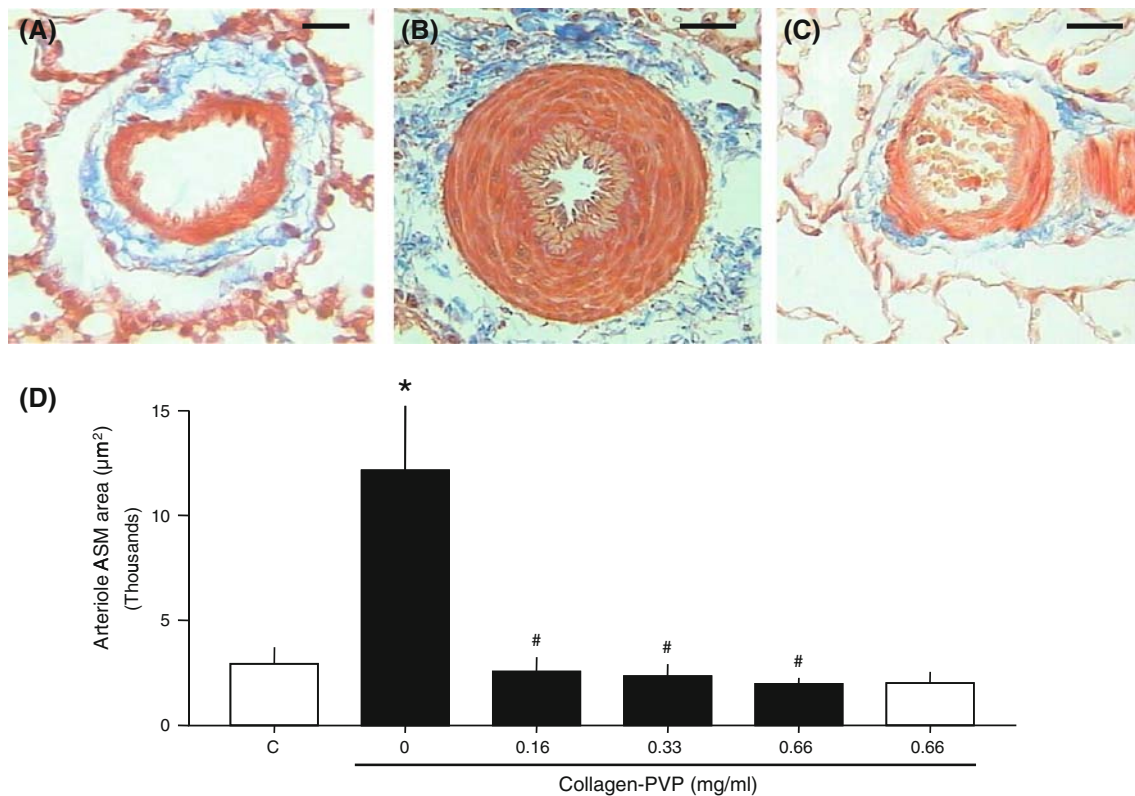


Fig. 3 Collagen-PVP effects on adjacent airway vessels in guinea pig allergic airways. Transversal view of a representative adjacent airway vessel from: (a) saline challenged, (b) ovalbumin challenged, and (c) ovalbumin challenged and 0.66 mg/ml collagen-PVP treated groups. Bar scales correspond to 30 μm. d Area of vascular smooth muscle (SM), adjusted by the basement membrane (BM) perimeter, as measured by automated morphometry. Bars and vertical lines are the

mean ± standard error of the mean for control and sham groups ($n = 7$ and 5 , respectively, white bars). Black bars represent ovalbumin-challenged groups ($n = 5$ all groups). * $P < 0.01$ compared with control group (one-way ANOVA and Dunnett's multiple comparisons test). # $P < 0.01$ compared with 0-mg/ml collagen-PVP ovalbumin-challenged group (one-way ANOVA and Tukey's multiple comparisons test). C control group

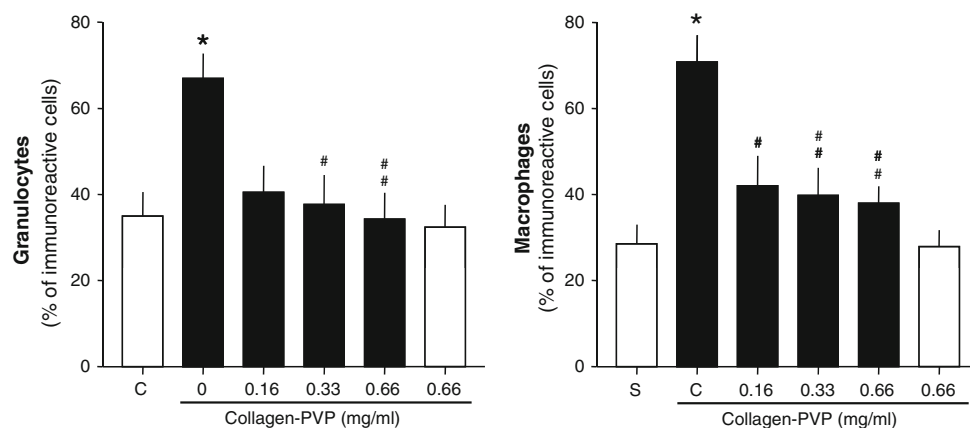


Fig. 4 Decrease of TNF-α immunoreactive leukocytes in allergic airway inflammation in guinea pigs. Percentage of granulocyte and macrophage TNF-α immunoreactive cells in airway wall. Bars and vertical lines are the mean ± standard error of the mean for control and sham groups ($n = 7$ and 5 , respectively, white bars). Black bars

represent ovalbumin-challenged groups ($n = 5$). * $P < 0.01$ compared with control group (one-way ANOVA and Dunnett's multiple comparisons test). # $P < 0.05$, ## $P < 0.01$ compared with 0-mg/ml collagen-PVP ovalbumin-challenged group (one-way ANOVA and Tukey's multiple comparisons test). C control group

group of asthma patients benefit from this therapy. Besides, malignancy incidence has increased in treated groups compared with placebo ones [31]. In this sense, it is

important to point out that collagen-PVP administration has produced no adverse effects during acute or chronic administration so far [11].

In this asthma model, collagen-PVP treatment reduced collagen accumulation in subepithelial mucosa. Fibrosis is a fundamental characteristic of airway remodelling during asthma; when fibrosis is advanced, reversal is not longer possible. Nevertheless, with an appropriate and mainly anti-inflammatory therapy, the advance of fibrosis can be reduced [32, 33]. We observed that collagen-PVP inhibited the development of fibrosis in our asthma model, and it is likely that collagen-PVP prevented subepithelial mucosa fibrosis by modulating airway inflammation.

Remodelling in asthma occurs not only in airways but also in adjacent structures. In rat and mouse asthma models, vessel enlargement has been observed after repetitive allergen challenge. In the present study, repetitive allergen challenge in guinea pigs induced pulmonary wall arteriole enlargement, suggesting that not only local but surrounding structures can be affected in this asthma model. Physiologically, vessel swelling can affect in vivo permeability and pulsatility, favoring cardiac dysfunction and hypertension, which is frequent in patients with asthma [34–36]. Inflammation has a central role in the development of hypertension [37]. We observed that collagen-PVP treatment prevented the wall vessel enlargement induced by repetitive allergen challenge, likely by controlling inflammation locally.

Barometric plethysmography is a method to assess pulmonary function with some advantages as the absence of animal restriction, the lack of pharmacological interference by anesthetic agents, and the possibility to make repetitive or prolonged studies without animal suffering [19]. Some studies in guinea pigs show that Penh is a good surrogate of specific airway resistance [38, 39]. In this study we did not find changes in Penh maximum peak induced by the antigen challenge. This suggests that collagen-PVP did not modify the magnitude of the antigen-induced airway obstruction.

Conclusions

Although the mechanisms involved in airway inflammation during asthma are still unknown, many factors contribute to alter the airway equilibrium. In this imbalance, TNF- α is one of the fundamental triggers of inflammatory response; inflammatory cells, such as eosinophils and neutrophils, are the apex in asthma phenotypes, and airway fibrosis plays an important role in asthma pathophysiology. By regulating many aspects of the altered airway environment produced by allergen challenges, collagen-PVP seems to be a promising asthma treatment because it has been shown to regulate different elements of the imbalanced airway environment produced by allergen challenges, as well as being safe and well tolerated.

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