



Influence of pirfenidone on airway hyperresponsiveness and inflammation in a Brown-Norway rat model of asthma

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Abstract

Pirfenidone was administered to sensitized Brown Norway rats prior to a series of ovalbumin challenges. Airway hyperresponsiveness, inflammatory cell infiltration, mucin and collagen content, and the degree of epithelium and smooth muscle staining for TGF- β were examined in control, sensitized, and sensitized/challenged rats fed a normal diet or pirfenidone diet. Pirfenidone had no effect on airway hyperresponsiveness, but reduced distal bronchiolar cell infiltration and proximal and distal mucin content. Statistical analysis showed that the control group and sensitized/challenged pirfenidone diet group TGF- β staining intensity scores were not significantly different from isotype controls, but that the staining intensity scores for the sensitized/challenged normal diet group was significantly different from isotype controls. These results suggest that pirfenidone treatment is effective in reducing some of the components of acute inflammation induced by allergen challenge.

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1. Introduction

For many years asthma was considered a reversible airways disease caused by autonomic nervous system-induced bronchospasm [1]. It has become clear, however, that long term changes occur in the airways of asthmatics, and a more complete picture of asthma has emerged where airway inflammation plays a central role with subsequent remodeling of the airway epithelium, submucosa, and mucosal layers [1–5]. Viewing asthma as a disease of recurring inflammation with subsequent remodeling in addition to acute episodes of reversible bronchospasm has lead researchers to adopt new therapies that focus on inflammation as the target for therapy [2]. The use of pirfenidone, an experi-

mental anti-inflammatory and anti-fibrotic drug with potent inhibitory effects on the production of TGF- β [6], may be one potentially new therapy employed against asthma.

Researchers have found a variety of changes that take place in the large and small conducting airways of human asthmatics [4] and in animal models of asthma. These changes include inflammatory cell influx [5,7,8], edema, airway wall thickening due to hypertrophy and hyperplasia of airway smooth muscle [5], basement membrane changes [3], goblet cell hyperplasia [7], and sub-epithelial fibrosis [7,9]. These changes are related, in turn, to the airway hyper-responsiveness that occurs in asthma [7,9]. A variety of inflammatory mediators are thought to be responsible for the airway remodeling in asthma. For example, interleukin-4 (Il-4), Il-5, Il-9, and Il-13, Th2-dominant inflammatory mediators, have been shown to be important in tissue eosinophilia,

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goblet cell hyperplasia, sub-epithelial fibrosis and airway hyper-responsiveness [1,3,5,8,10] in animal models of asthma. Transforming growth factor-beta (TGF- β), thought to play an important role in the sub-epithelial fibrosis found in the asthmatic airway [11–13], is produced by airway epithelial cells, eosinophils, Th2 cells, macrophages, and fibroblasts [14], and is elevated in bronchoalveolar lavage fluid and biopsies [1,12,13,15] of asthmatics. Transforming growth factor-beta has also been shown to be released from airway smooth muscle cells, and to induce those cells to synthesize procollagen I [16]. The elevation in TGF- β in asthma may be a consequence of a TGF- β promoter polymorphism resulting in increased TGF- β transcriptin [14,17,18]. To date, no drug therapies specific for TGF- β have been utilized in the treatment of asthma.

Pirfenidone [5-methyl-1phenyl-2-(1H)-pyridone; trade-name Deskartm] has been shown to be an effective anti-fibrotic drug [6,19–21] in a bleomycin model of pulmonary fibrosis in hamsters as shown by decreases in lung hydroxyproline content [21]. Additionally, pirfenidone has been shown to be effective in reducing collagen build-up in rat models of liver [22] and kidney [23] fibrosis, in a rat model of sclerosing peritonitis [24] and in a model of glomerulosclerosis in mice [25]. Both rat [26] and human [27] studies have shown few side-effects of this drug. In addition, pirfenidone has potent anti-inflammatory effects [20,28] as shown by its ability to inhibit tumor necrosis factor-induced endotoxin shock [29] and TGF- β gene expression [6]. Pirfenidone, however, has never been used in a subacute animal model of asthma or human asthmatics. In the first study of its kind, we used pirfenidone in a Brown Norway rat model of subacute allergic inflammation to examine its effect on a variety of markers of allergic airway disease. We hypothesized that pirfenidone treatment would reduce measures of allergic airway disease including airway hyperresponsiveness, inflammation and remodeling. We examined the effect of pirfenidone on airway hyperresponsiveness, inflammatory cell infiltration, mucin and collagen content, and TGF- β immunoreactive staining. Although pirfenidone had no effect on airway hyper-responsiveness, pirfenidone significantly reduced distal bronchiolar cellularity and proximal and distal bronchiolar mucin content. Pirfenidone treatment also showed indications of an inhibitory effect on TGF- β production in airway epithelium and smooth muscle lung sections.

2. Materials and methods

2.1. Study design and animals

Forty-eight 8 weeks old male Brown Norway rats (BN/SsNHsd) obtained from a pathogen free colony (Harlan Sprague Dawley, Prattville, AL) were used in this study. The animals were housed in inhalation chambers ventilated with filtered air. Food and deionized filtered water were provided ad libitum, and rats were acclimated for 1 week prior to the start of the experiment. Two sentinel rats were placed in the inhalation chambers for the duration of the study in order to guard against possible infection of the colony by pathogens. The sentinel rats were sent to the Comparative Pathology Laboratory at University of California, Davis for health screening to confirm the absence of respiratory pathogens, virus antibodies and parasites. Comprehensive health screening included microbiology, histopathology and serology indicated that the rats were free of known pathogenic agents. One non-sentinel rat died in the sensitized/challenged pirfenidone diet group during the course of sensitization from undetermined causes (Table 1).

The rats were randomly assigned to one of six groups: two control groups, two sensitized groups and two sensitized/challenged groups. Three of the groups ingested a normal diet of pulverized rat chow, and the other three groups ingested a diet consisting of pulverized rat chow with 0.5% (wt/wt) pirfenidone (5-methyl-1phenyl-2-(1H)-pyridone; tradename Deskartm; Marnac, Inc., Dallas, TX). The animals were placed on the pirfenidone diets 10 days after sensitization (Fig. 1). The dose of pirfenidone used in this study was chosen based on the dose that has been effective in a bleomycin model of lung fibrosis that inhibited both excessive collagen build up and TGF- β in the lung [6,21].

2.2. Induction of allergic airway disease

Allergic airway disease was induced in the same manner as Larson et al. [30]. Rats were sensitized with a subcutaneous injection of 1 mg chicken ovalbumin (Sigma-Aldrich, St. Louis, MO; agarose electrophoresis purified, >99% pure) and 200 mg aluminum hydroxide (Sigma-Aldrich, St. Louis, MO) in 1 ml of sterile saline, and 1 ml of *Bordetella pertussis* vaccine (Bioport Corporation,

Table 1

Research design examining the effect of pirfenidone on Brown Norway rats sensitized and challenged with ova albumin

Control		Sensitized		Sensitized/challenged	
Sham sensitization + filtered air		Sensitization + filtered air		Sensitization + ova albumin challenges	
Normal diet	Pirfenidone diet	Normal diet	Pirfenidone diet	Normal diet	Pirfenidone diet
Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
n = 8	n = 8	n = 8	n = 8	n = 8	n = 7

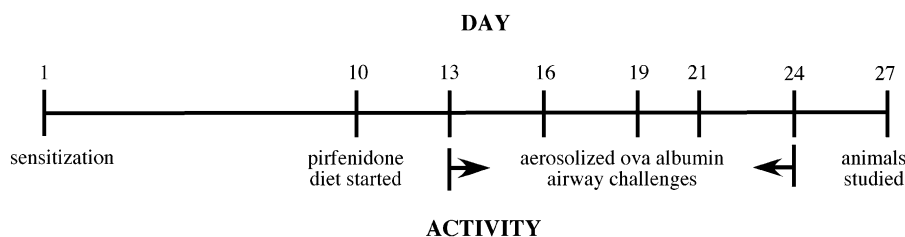


Fig. 1. Research activity timeline for diet and treatment.

Lansing, MI) containing 6×10^9 heat-killed organisms injected intraperitoneal (IP). Sham sensitized rats received subcutaneous and IP saline injections only. Twelve days later rats received their first aerosolized ovalbumin challenge. A total of five ovalbumin challenges were administered 3 days apart (Fig. 1). Sham challenged animals received filtered air.

For a detailed description of aerosol generation see Larson et al. [30]. Aerodynamic size distributions were determined using a seven-stage Mercer-type cascade impactor (ARIES, Inc., Davis, CA) [31]. The content of chloride anion derived from saline residue in the particles was measured on each impactor stage and the after-filter by ion chromatography (Model DX-120; Dionex Corp., Sunnyvale, CA). A log-normal distribution was fitted to each sample set of data, and the values reported are the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (σ_g) of the fitted distributions. The total mass concentration of the aerosol was $12.63 \pm 0.84 \text{ mg/m}^3$ ($n = 10$), and the protein content was $6.9 \pm 1.19 \text{ mg/m}^3$ ($n = 6$). The mass MMAD was $1.93 \pm 0.15 \mu\text{m}$ ($n = 3$) with a σ_g of 1.99 ± 0.08 . Each ovalbumin aerosol exposure was conducted for 30 min after allowing 18 min for chamber equilibration to 99% of the final concentration. All aerosol data is mean $\pm$ standard deviation.

2.3. Measurement of airway hyperresponsiveness

The animals were studied 3 days after the last ovalbumin airway challenge. Animals were anaesthetized with mixture of α -chloralose and urethane (0.1 g α -chloralose/kg body weight, 10 g urethane/kg body weight IP) and intubated with a 14 gauge catheter. Changes in airway responsiveness were examined by delivering aerosolized methacholine for 1 min and measuring the change in airway resistance for the next 3 min. This was accomplished by the rats being placed in a whole body plethysmograph and their tracheal cannula attached to a constant volume positive pressure ventilator (model 683, Harvard Inc.) set at a tidal volume of 0.7 ml/100 g body weight at a frequency of 90 breaths/min. The rats were then paralyzed with succinyl chloride (0.2 ml at 20 mg/ml IP) and the plethysmograph was closed. Air flow was measured using a pneumotachograph (model 8300, Hans Rudolph Inc.) mounted into the side of the plethysmograph. Airway pressure was measured using a differential pressure transducer (Validyne DP15,

Northridge, CA) with one side attached near the tracheal cannula port of the ventilator and the other side attached to a fluid filled cannula with side ports near the tip and whose tip was placed in the esophagus in the mid-thoracic region. The air flow and airway pressure signals were amplified and then recorded and analyzed using a digital data acquisition system (PO-NE-MAH, Gould Instrument Systems, Inc., Valley View, OH). Pulmonary flow resistance (R_L) was calculated according to the method of Amdur and Mead [32] with air flow and airway pressure being measured at an isovolume of 70% of tidal volume. A starting dose of 0.125 mg/ml methacholine was used and continued in doubling doses until airway resistance had doubled or a concentration of 64 mg/ml methacholine had been reached. The concentration of methacholine required to double lung resistance (EC200 R_L) was obtained by linear interpolation between the two concentrations bounding the point at which lung resistance reached 200% of control on a log–log plot. Immediately following airway responsiveness evaluation the lungs were removed and fixed at a volume of 30 cm H_2O using zinc-formalin (Z-fix, Anatech, Battle Creek, MI).

2.4. Assessment of lung histology

Following fixation of the lungs by intratracheal instillation of formalin (Z-fix) at a pressure of 30 cm H_2O , tissue sections were prepared by cutting transverse lung slices immediately cranial and caudal to the hilum of the left lobe. Each tissue slice was embedded in paraffin and sectioned using a Microm HM 355 rotary microtome (Zeiss, Thornwood, NY). Three distinct anatomical regions from the lungs of each animal were examined: (1) the main axial airway path of the left caudal lobe (referred to as proximal airway), (2) the general pulmonary vasculature, and (3) the terminal bronchiole and alveolar duct (referred to as distal airway). All sections were cut 5 μm thick. Serial tissue sections were stained with hematoxylin and eosin (H&E) to observe general pulmonary structures, alcian blue/periodic acid Schiff (AB/PAS) for epithelial distribution of mucin, sirius red for collagen and basement membrane features and combined eosinophil/mast cell (CEM) stain for visualization of eosinophils and mast cells.

To define the general features of the central airways in the Brown Norway rat lung, the main axial airway pathway of the left caudal lobe was examined. This airway was examined in cross-section at the level of the third to

sixth generation to confirm that the same general airway site would be described for all animals studied. In contrast, using a process of random field generation for each tissue section [33,34], a total of 10 blood vessels, five terminal bronchioles and five parenchymal regions immediately arising from terminal bronchioles were examined to ensure an unbiased analysis of these anatomic features in the Brown Norway rat. Blood vessels that appeared in cross-section and were greater than 75 μ m in diameter were examined. Both arteries and veins were combined in the analysis. Terminal bronchioles were identified in tissue sections as airways directly opening into alveolar-lined ducts.

Airway mucin was measured for all airways. Weakly acidic sulfated mucosubstances stained turquoise, while mucosubstances containing glycol groups stained magenta [35]. For each airway, images were collected using a 10X objective with an Olympus BH2 microscope interfaced to a computer with a videocamera (Dage MTI, Michigan City, IN). The abundance of AB/PAS positive mucosubstances within the epithelium was determined by area analysis using NIH Image program (NIH Image, Bethesda, MD) with the density gradient feature to highlight positively stained areas of the epithelium. All measurements were normalized to the basal lamina length squared of each airway.

Collagen volume was measured for each airway using a 550 nm filter to enhance visualization of sirius red (excitation maximum wavelength approximates 550 nm) stained substances. These measurements were expressed per basal lamina length squared of each airway. The relative abundance of eosinophils and mast cells within the walls of airways and blood vessels were measured as the number of cells per basal lamina length squared. For airways, both cells present within the epithelial layer as well as within the interstitial wall were combined.

The relative cellularity of the blood vessel wall as well as the centriacinar regions of the lungs defined as those areas of parenchyma immediately arising from terminal bronchioles was based on a semi-quantitative scoring system. A score of 0 was given to normal structures with no significant changes, a score of 1 was given to scattered, random, mixed inflammatory cells, a score of 2 was given to moderate numbers of mixed inflammatory cells, and a score of 3 was given to clusters of mixed inflammatory cells indicating severe cellular influx [36]. All tabulations of airway cellularity were performed in a blinded fashion.

2.5. TGF- β immunohistochemistry and assessment

The lower left lobe paraffin blocks were serially sectioned at 5 μ m and used for TGF- β immunohistochemistry. The sections were deparaffinized in a xylene and graded alcohol series and incubated in 1% goat serum in 0.1 M phosphate buffered saline at a pH of 7.4 for 60 min to block nonspecific binding. Excess fluid was blotted and sections were incubated overnight in a humidified chamber

at 4 °C in 1:400 mouse monoclonal anti-human TGF- β antibody (Serotec, Raleigh, NC) and 1% bovine serum albumin (BSA) or purified mouse IgG as an isotype control (Chemicon, Australia). The sections were again incubated in 1% goat serum in 0.1 M phosphate buffered saline at a pH of 7.4 for 60 min. Subsequently, slides were incubated for 1 h with secondary antibody, Alexa 568 goat anti-mouse (Molecular Probes, Eugene, OR) antibody. Slides were subsequently mounted in Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA).

The largest conducting airway present in each section was visualized by confocal microscopy (Radiance 2100 Confocal System) using a 40 $\times$ oil immersion lens. Images were captured from the same region of the airway wall for both TGF- β antibody and isotype control from serial sections using the same microscope and camera settings. The 14 images of the TGF- β stained slides were then compared with their 14 isotype controls and the degree of staining of the epithelium and airway smooth muscle was scored on a scale from 0 (no difference in intensity between the TGF- β and isotype control image) to 10 (major difference in intensity between the TGF- β and isotype control image) by a blinded examiner.

2.6. Statistics

Data was analyzed using a two-way ANOVA (Statview, SAS institute, Cary, NC) with diet (control, pirfenidone) and treatment (control, sensitized, sensitized/challenged) as independent variables. Post hoc analysis was done using Fisher's PLSD. Significance was set at $p \leq 0.05$. All data is reported as averages $\pm$ standard errors unless otherwise stated. A one sample sign test was used to determine if TGF- β staining intensity scores were different from zero.

3. Results

The average daily dose ($\pm$ standard deviation) of pirfenidone ingested was 264.4 ± 41.1 mg/kg/d. There were no significant differences in the body weight of the rats placed on the pirfenidone diet compared with the normal diet indicating that the rats tolerated this dose of pirfenidone.

3.1. Airway responsiveness to methacholine

The effective concentration of methacholine at which airway resistance doubled (EC_{200R_L}) was significantly reduced in the sensitized/challenged groups compared with the control and sensitized groups (Fig. 2). There was no significant difference in EC_{200R_L} between the control and sensitized groups. Additionally, EC_{200R_L} for the groups that received pirfenidone in their diets was not significantly different than the groups that received the normal diet.

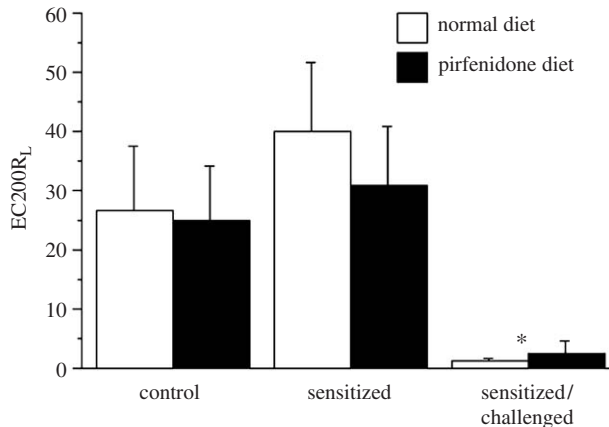


Fig. 2. Effective concentration of methacholine at which airway resistance doubled (EC200RL). *Sensitized/challenged normal diet and pirfenidone diet groups significantly reduced from control and sensitized groups.

3.2. Proximal and distal airway collagen content

Mid-level segmental bronchi (proximal airway) and terminal bronchiole (distal airway) collagen content was examined between the control groups and sensitized/challenged groups. The sensitized groups were not a part of this analysis. There were no significant differences between these treatment groups either in the proximal or distal airways, nor were there any collagen content differences between the groups that received the pirfenidone and the normal diets at either airway site (data not shown).

3.3. Proximal and distal airway inflammatory cell infiltration

Proximal airway eosinophil and mast cell numbers relative to basal lamina length squared was examined in all groups. There was a significant increase in the number of eosinophils in the sensitized/challenged groups compared with the control and sensitized groups in the proximal airways (Fig. 3A). There was also a significant increase in the number of mast cells in the sensitized/challenged groups compared with the control groups in the proximal airways (Fig. 3B). There was no effect of pirfenidone ingestion on proximal airway cell infiltration.

Distal airway cell infiltration was examined on a 0–3 subjective scale with 0 representing normal cellularity and 3 representing severe cellularity. There was a significant increase in cellularity in the sensitized/challenged normal diet group compared to the control and sensitized groups (Fig. 4). There was also a significant increase in cellularity in the sensitized/challenged pirfenidone diet group compared with its control group. Post hoc analysis showed that the cellularity in the distal airways of the sensitized/challenged group that received the pirfenidone diet was significantly less than the cellularity of the sensitized/challenged group that received the normal diet, signifying

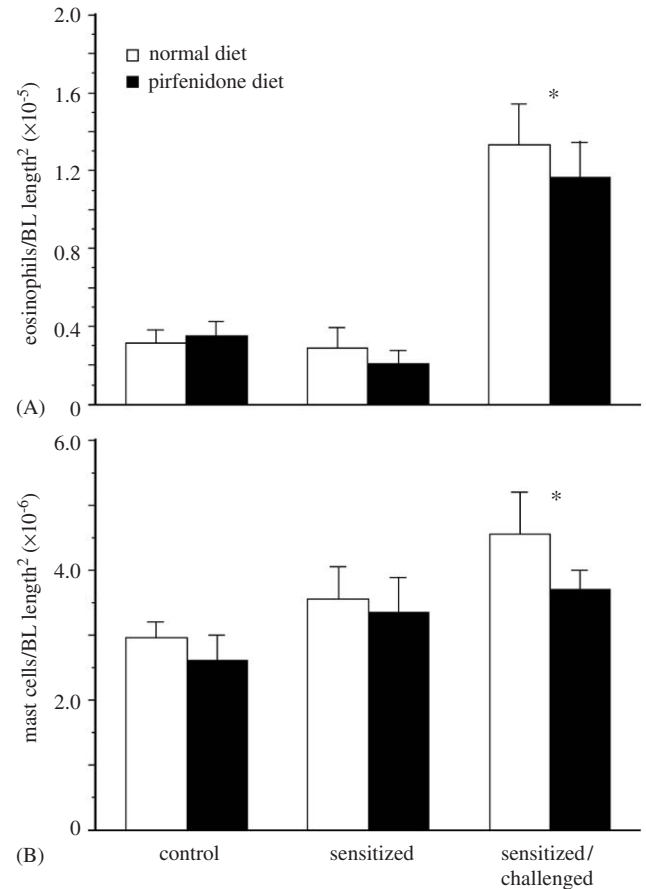


Fig. 3. Proximal airway cell infiltration of eosinophils (A) and mast cells, (B) per length of basal lamina (BL) squared. *Sensitized/challenged normal diet and pirfenidone diet groups significantly increased from control and sensitized groups (A) and from control groups (B).

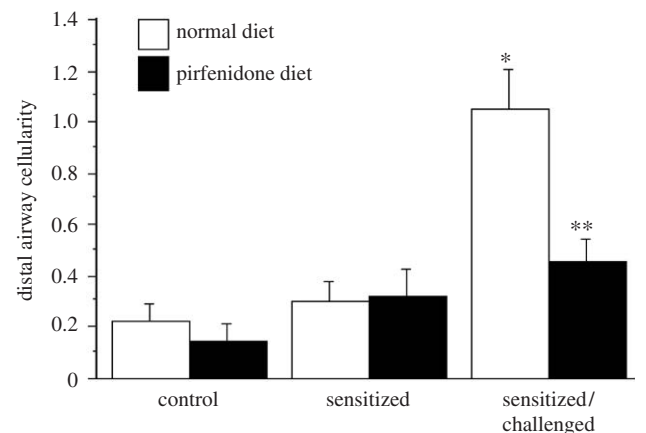


Fig. 4. Distal airway cell infiltration (cellularity). Airway cellularity was determined in a blinded fashion using a 0 (normal structures, no influx of cells) to 3 (clusters of mixed inflammatory cells, severe influx of cells) scale. *Sensitized/challenged normal diet group significantly greater than normal diet and pirfenidone diet control and sensitized groups; **sensitized/challenged pirfenidone diet group significantly reduced from sensitized/challenged normal diet group.

the effectiveness of pifenidone in inhibiting distal airway cellular infiltration in the terminal bronchioles of sensitized/challenged rats.

3.4. Proximal and distal airway mucin levels

Proximal and distal airway mucin area relative to basal lamina length squared was examined in all groups. In both the proximal (Fig. 5A) and distal (Fig. 5B) airways, the sensitized/challenged normal diet and pifenidone diet groups mucin areas were significantly greater than the control or sensitized groups. In addition, pifenidone ingestion significantly reduced mucin area in both the proximal and distal airways in the sensitized/challenged groups, indicating pifenidone's effect on mucous cell metaplasia or mucin volume per cell.

3.5. Proximal airway epithelium and smooth muscle TGF- β staining

We observed TGF- β positive epithelium and smooth muscle (Fig. 6A–F) within the midlevel segmental bronchi (proximal airways) that was higher in staining intensity in

the sensitized/challenged normal diet group (mean: 4.71; range: 0–12) compared with either the control (mean: 2.00; range: 0–8) or the sensitized/challenged pifenidone diet group (mean: 4.50; range: 0–9). The results of the one sample sign tests showed that the control and sensitized/challenged pifenidone diet groups staining intensity scores were not significantly different from zero, but that the staining intensity score for the sensitized/challenged normal diet group was significantly different from zero. This indicates that the blinded evaluator observed greater overall staining intensity relative to the isotype controls in the sensitized/challenged normal diet group compared with the control and sensitized/challenged pifenidone diet groups. However, although statistically significant, these results must be viewed with caution as there was large variability in the scores.

4. Discussion

This is the first study that we are aware of that examines the effect of pifenidone supplementation on lung functional and morphological changes in the Brown Norway rat model of asthma [37]. We are confident that we induced significant allergic airway disease in this rat model as indicated by numerous differences in both functional and morphological indices between our control and sensitized/challenged groups. Although we saw no changes in airway responsiveness to methacholine challenge in rats fed pifenidone prophylactically, pifenidone treatment did reduce distal airway cell infiltration and proximal and distal mucin content. In addition, pifenidone treatment showed indications of an inhibitory effect on TGF- β production in airway epithelium and smooth muscle lung sections. These results indicate that pifenidone treatment is effective in reducing the acute inflammation induced by allergen challenge which may be in part dependent upon TGF- β . The reduction in TGF- β positive airway epithelium and smooth muscle suggests that pifenidone may also act as a possible anti-fibrotic agent [6,16] in a more chronic model of asthma. Pifenidone, however, seems to be having its greatest effect on the acute inflammatory components of this allergic airway model.

A variety of changes occurred in rats sensitized with ovalbumin/*Bordetella pertussis* and challenged with aerosolized ovalbumin indicating that our Brown Norway rat model of asthma was both functionally and morphologically similar to human asthma. Human asthmatics exhibit airway hyperresponsiveness to methacholine challenge indicated by significant reductions in FEV₁ and increases in airway resistance [13]. Our sensitized and challenged rats showed significant decreases in the effective concentration at which airway resistance doubled in agreement with Bellofiore et al. [10], Du et al. [3], and Sapienza et al. [5]. In addition, our sensitized/challenged rats showed significant increases in eosinophils and mast cells in the proximal airways that were not effected by pifenidone as well as increases in terminal bronchiolar cellularity compared with

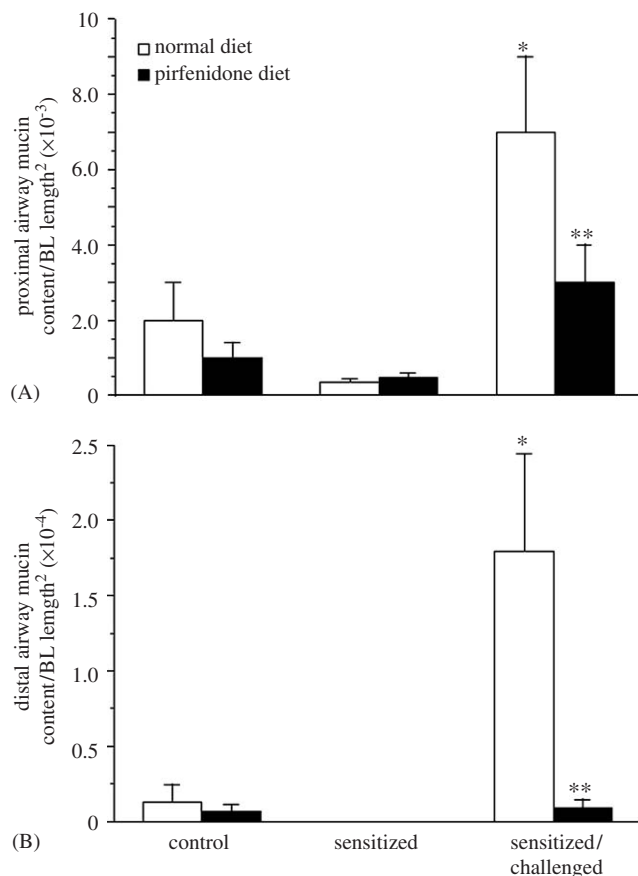


Fig. 5. Proximal (A) and distal (B) airway mucin content per length of basal lamina (BL) squared. *Sensitized/challenged normal diet group significantly greater than normal diet and pifenidone diet control and sensitized groups; **sensitized/challenged pifenidone diet group significantly reduced from sensitized/challenged normal diet group.

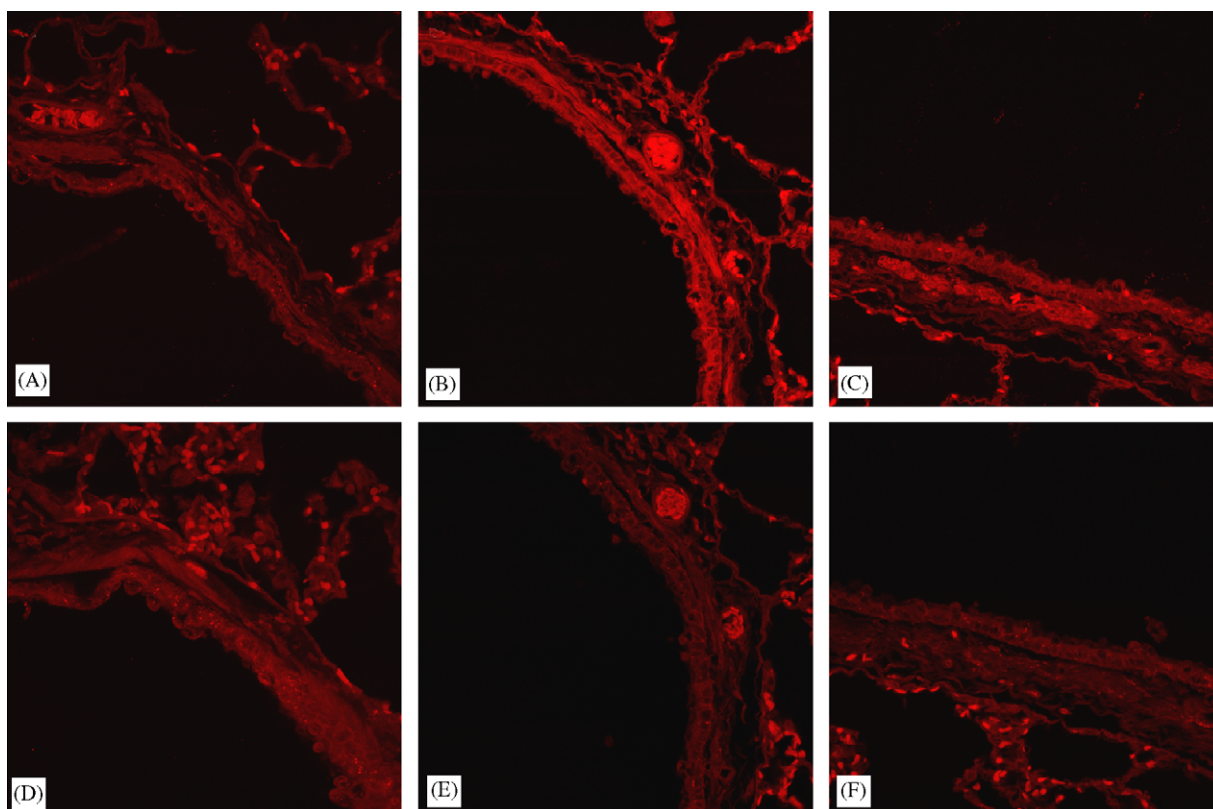


Fig. 6. Proximal airway sections stained for TGF- β (A,B,C) and isotype control (D,E,F). Control (A,D), sensitized/challenged + normal diet (B,E), sensitized/challenged + pirfenidone diet (C,F).

the control groups. Human asthmatics have been shown to have increased eosinophils in bronchoalveolar lavage [1] and in airways during biopsy [13] and necropsy [4]. Mucin levels were also increased in our sensitized/challenged rats compared with control rats in both proximal and distal airways, again mimicking human asthma [1] and in agreement with Palmans et al. [7] in a Brown Norway rat model of asthma. The observation that pirfenidone treatment reduced distal airway cell infiltration and distal mucin content relatively more than proximal mucin content may indicate that distal parts of the lung supplied by the pulmonary circulation may be exposed to more pirfenidone than the proximal airways vascularized by the bronchial circulation.

The observation of greater TGF- β intensity in airway epithelium and smooth muscle in sensitized/challenged rats indicates that the duration of allergen exposure used in this experiment was sufficient to initiate an important pathway in the development of airway inflammation and fibrosis [16,38]. Unfortunately, we did not see any significant changes in airway collagen content which occurs in human asthma [9] and was shown by Palmans et al. [7] to occur in a more chronic Brown Norway rat model of asthma. The lack of peribronchial fibrosis in our experimental animals is most likely due to the relatively short amount of time the animals were exposed to allergen (2 weeks) as compared to allergen exposure of up to 12 weeks in the Palmans et al. [7] study. However, the observation that pirfenidone treat-

ment reduced the intensity of proximal airway epithelium and smooth muscle staining for TGF- β suggests that this treatment may be effective in preventing the airway fibrosis observed in a more chronic model of allergic airway disease [7].

The changes in cellular influx, mucin content and the reduction of TGF- β intensity in airway epithelium and smooth muscle in the animals treated with pirfenidone implies that pirfenidone is partially effective in reducing the acute inflammatory response to airway challenge and is potentially effective in inhibiting some of the chronic components of asthma. Pirfenidone is known to inhibit TGF- β [6], a known chemo-attractant to monocytes, macrophages [39], and fibroblasts [40]. These cells, in turn, can recruit other inflammatory cell types through release of their inflammatory cytokines. Additionally, pirfenidone has been shown to down-regulate intercellular adhesion molecules [41] that may be important in inflammatory cell influx into the lung. Interestingly, ingestion of pirfenidone did not reduce airway hyperresponsiveness to methacholine present in ovalbumin sensitized/challenged rats. This observation indicates that the increases in TGF- β and/or the proximal and distal airway cellular infiltration and mucin content that were significantly reduced by pirfenidone treatment are not directly associated with the development of airway hyper-responsiveness in this model of allergic airway disease. In conclusion, our observations support the need for further studies examining the

effectiveness of pirfenidone on both the acute and chronic components of allergic airway diseases such as asthma.

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