

Investigating the Role of Relaxin in the Regulation of Airway Fibrosis in Animal Models of Acute and Chronic Allergic Airway Disease

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ABSTRACT: Airway remodeling is a characteristic feature of asthma that leads to chronic irreversible airway obstruction. Fibrosis in the basement membrane region is a hallmark of remodeling in asthma that is not found in other diseases. In the outlined studies, we investigated the relationship between relaxin and airway fibrosis in asthma using acute and chronic models of allergic airway disease. These studies confirm a critical role for relaxin, in the regulation of collagen deposition in the airway/lung in animal models of allergic airway disease.

KEYWORDS: relaxin; airway/lung fibrosis; relaxin-1 knockout mice; acute and chronic allergic airway disease; asthma

INTRODUCTION

Airway remodeling is a hallmark of asthma and is characterized by increased deposition of collagen (fibrosis) in the subepithelial basement membrane (BM) region, smooth muscle hypertrophy and hyperplasia, fibroblast hyperplasia, epithelial metaplasia, and goblet cell proliferation.¹ However, the factors regulating collagen turnover have not been clearly defined. Consequently, there are currently no treatments that effectively reverse established collagen deposition in asthma.

Relaxin (RLX) is well known for its ability to stimulate the breakdown of collagen and was shown to inhibit collagen accumulation in a bleomycin-induced model of lung fibrosis,² by inhibiting transforming growth factor- β -induced production of matrix proteins and stimulating matrix metalloproteinase-induced collagen breakdown. Furthermore, RLX-deficient mice were shown to develop an age-related

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progression of fibrosis in the airway/lung,³ a phenotype that could be reversed by recombinant H2 relaxin treatment. These findings suggest an important role for RLX in regulating collagen deposition within the lung.

In the present study, we examined the role of RLX in the regulation of collagen deposition during an allergic inflammatory response, using RLX-deficient mice and models of allergic airway disease.

ROLE OF RELAXIN IN AIRWAY REMODELING IN A CHRONIC MODEL OF ALLERGIC AIRWAY DISEASE

An ovalbumin (OVA)-induced model of allergic airway disease (AAD) was applied in 2-month-old wild-type (RLX^{+/+}) and RLX-deficient (RLX^{-/-}) mice, with similar baseline collagen levels. The animals were sensitized to either 0.5 mL saline or OVA (10 µg) in 0.5 mL of saline on days 0 and 14, before being challenged with aerosols of either saline or 2.5% (w/v) OVA, 3 days a week (from days 21 to 63), for a 30-min period each day. On day 64, 24 hours after the last saline or OVA challenge, RLX^{+/+} and RLX^{-/-} mice were assessed by unrestrained⁴ and invasive plethysmography, before the animals were killed and lung tissue was isolated and analyzed for various parameters.

In the chronic model of AAD, RLX^{-/-} mice were able to mount similar inflammatory responses after OVA sensitization and challenge, to that of RLX^{+/+} mice, as demonstrated by an increase in OVA-specific IgE levels and influx of inflammatory cells into the airways/lung. Furthermore, RLX^{-/-} mice mounted similar OVA-induced AHR responses to that of RLX^{+/+} mice. OVA treatment of RLX^{+/+} mice induced modest increases in lung collagen deposition and OVA treatment of RLX^{-/-} mice significantly enhanced collagen deposition ($P < .05$ vs. saline-treated groups). Other changes included increased airway fibrosis and decreased MMP-2 and MMP-9 expression ($P < .05$ vs. OVA-treated RLX^{+/+} mice).

ROLE OF RELAXIN IN AIRWAY REMODELING IN A SUBACUTE MODEL OF ALLERGIC AIRWAY DISEASE

Twelve-month-old RLX^{+/+} and RLX^{-/-} mice with established pulmonary fibrosis were sensitized to either 0.5 mL saline or OVA (10 mg) in 0.5 mL of saline on day 0, before being challenged with aerosols of either saline or 1% (w/v) OVA on days 14–20, for a 30-min period. On day 21, 24 hours after the last saline or OVA challenge, unrestrained RLX^{+/+} and RLX^{-/-} mice were assessed for methacholine-induced airway reactivity, using whole-body plethysmography,⁴ before the animals were killed and lung tissue was isolated and analyzed for various parameters.

In the subacute model of AAD, RLX^{-/-} mice were also able to mount similar inflammatory responses after OVA sensitization and challenge, to that of RLX^{+/+} mice, as just detailed. However, saline-treated RLX^{-/-} mice had increased lung collagen content/deposition, increased bronchiole epithelial thickness, and increased airway hyperresponsiveness (AHR) (all $P < .05$) as compared with that in saline-treated RLX^{+/+} mice. Furthermore, OVA-treated RLX^{-/-} mice developed exaggerated collagen content/deposition in the airway wall ($P < .05$ vs. saline-treated and

OVA-treated RLX^{+/+} mice) and a further significant thickening of the epithelial ($P < .05$ vs. all other groups) and BM regions ($P < .05$ vs. saline-treated groups). AHR remained elevated and was equivalent to that in saline-treated RLX^{-/-} mice and OVA-treated RLX^{+/+} (all $P < .05$ vs. AHR values from saline-treated RLX^{+/+} mice).

CONCLUSIONS

These combined findings demonstrate that relaxin is a naturally occurring inhibitor of collagen turnover in the lung and appears to play a critical role in protecting the lung from increased fibrosis, associated with the adverse effects of acute and chronic AAD.

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