



Roxithromycin suppresses airway remodeling and modulates the expression of caveolin-1 and phospho-p42/p44MAPK in asthmatic rats



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ABSTRACT

Roxithromycin (RXM) expresses anti-asthmatic effects that are separate from its antibiotic activity, but its effects on airway remodeling are still unknown. Here, we evaluated the effects of RXM on airway remodeling and the expression of caveolin-1 and phospho-p42/p44mitogen-activated protein kinase (phospho-p42/p44MAPK) in chronic asthmatic rats. The chronic asthma was induced by ovalbumin/Al(OH)₃ sensitization and ovalbumin challenge, RXM (30 mg/kg) or dexamethasone (0.5 mg/kg) was given before airway challenge initiation. We measured the thickness of bronchial wall and bronchial smooth muscle cell layer to indicate airway remodeling, and caveolin-1 and phospho-p42/p44MAPK expression in lung tissue and airway smooth muscle were detected by immunohistochemistry and western blot analysis, respectively. The results demonstrated that RXM treatment decreased the thickness of bronchial wall and bronchial smooth muscle cell layer, and also downregulated the phospho-p42/p44MAPK expression and upregulated the caveolin-1 expression. The above effects of RXM were similar to dexamethasone. Our results suggested that pretreatment with RXM could suppress airway remodeling and regulate the expression of caveolin-1 and phospho-p42/p44MAPK in chronic asthmatic rats.

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1. Introduction

As a characteristic feature of asthma, airway remodeling was formerly regarded as a consequence of chronic airway inflammation. But recent studies have demonstrated that remodeling can occur in the early asthmatic process and airway inflammation [1,2]. Among a variety of airway structural changes, airway smooth muscle hyperplasia was considered as the major pathological change and would be established as the “target” for asthmatic airway remodeling [3–5].

Caveolae are micro structures which form flask-shaped invaginations of the plasma membrane and are involved in the process of cell signal transduction [6]. Caveolin-1 constitutes the principal protein component of caveolae and expresses in smooth muscle cells in particular in the respiratory tract. The reduced expression of caveolin-1 has been demonstrated to be associated with the activation of transforming growth factor- β signaling, which plays an important role in the development of airway remodeling in various conditions [7]. Therefore,

caveolin-1 could be considered as a potential key regulatory protein in the process of chronic airway inflammatory diseases including asthma and chronic obstructive pulmonary disease [8]. Moreover, caveolin-1 could interact with many signal molecules through special scaffolding domain [9], and was associated with the suppression of MAPK signaling pathway [10]. siRNA-knockdown of caveolin-1 caused spontaneous p42/p44MAPK activation [11]. p42/p44MAPK, a crucial effector of cell proliferation, may be involved in the regulation of airway smooth muscle proliferation [11–13].

Roxithromycin (RXM), a new 14-membered macrolide antibiotic, is effective for diffuse panbronchiolitis and asthma [14,15]. It may be related to its immunomodulatory, anti-inflammatory and anti-oxidative activities independent on its antibacterial action. By regulating MAPK/nuclear factor- κ B pathway in inflammatory cells and downstream inflammatory cytokines, RXM could inhibit airway inflammation and hyperresponsiveness in ovalbumin-specific allergic asthma [16]. However, the effects of RXM on airway remodeling in chronic asthmatic process remain unclear.

In this present study, we hypothesized that RXM may regulate the expression of caveolin-1 and phospho-p42/p44MAPK and attenuate airway remodeling in chronic asthmatic rats. We used a rat model of chronic asthma induced by Al(OH)₃ and ovalbumin, and the airway wall area, smooth muscle wall area and basement membrane perimeter were employed to indicate airway remodeling. Caveolin-1 and

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phospho-p42/p44MAPK expressions were measured by immunohistochemistry and western blot methods, also the effects of both RXM and dexamethasone were evaluated.

2. Methods

2.1. Group allocation

The study was approved by the Animal Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University. Thirty-two male Sprague Dawley rats, 5-week-old, weighing 100–120 g, were randomized into four groups: asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D) with 8 rats in each group. Chronic asthma model in Groups A, R and D was established as the improved method by Palmans et al. [17]. The rats were sensitized by intra-peritoneal injection of 1 mL solution of ovalbumin 1 mg and $\text{Al}(\text{OH})_3$ 100 mg on day 1 and day 7. From day 15 after the initial sensitization, the rats received an airway challenge with 8 mL 1% ovalbumin (diluted with normal saline) for 0.5 h, qod for 8 weeks, using an ultrasonic nebulizer. Intragastric administration of RXM (30 mg/kg) and intraperitoneal injection of dexamethasone (0.5 mg/kg) were given half an hour before the airway challenge initiation in Groups R and D, respectively; the remaining treatments were identical to Group A. Rats in Group C were injected intraperitoneally with normal saline and inhaled aerosolized normal saline at the same

time as the other three groups. After the 8-week airway challenge, lung tissue and bronchial smooth muscle cell layer of each group were collected in 24 h.

2.2. Preparation of HE staining

Thin slices of lung tissue were fixed in 4% formaldehyde solution for 5–6 h and were processed routinely for paraffin embedding. Then 4 μm -thick sections were cut and stained with hematoxylin and eosin. The total wall area (Wat), smooth muscle wall area (Wam) and basement membrane perimeter (Pbm) were measured by Image-Pro Plus software 6.0 calibrated with a reference micrometer slide. Five images were analyzed per section at 200 $\times$ magnification. Wat/Pbm and Wam/Pbm were used for indicating the thickness of bronchial wall and bronchial smooth muscle cell layer respectively, and a schematic diagram was provided as Fig. 2A.

2.3. Preparation of transmission electron microscopy (TEM)

1 mm³ tissues were collected from left lung tissue of each group, which were then prefixed in 2.5% glutaraldehyde solution overnight at 4 °C. After being washed three times with 0.1 M sodium phosphate buffer solution, samples were postfixed in 1% osmium tetroxide for 1 h at 37 °C, then washed again with phosphate buffer solution and stained with 1% uranyl acetate for 1 h at 37 °C. After this, samples

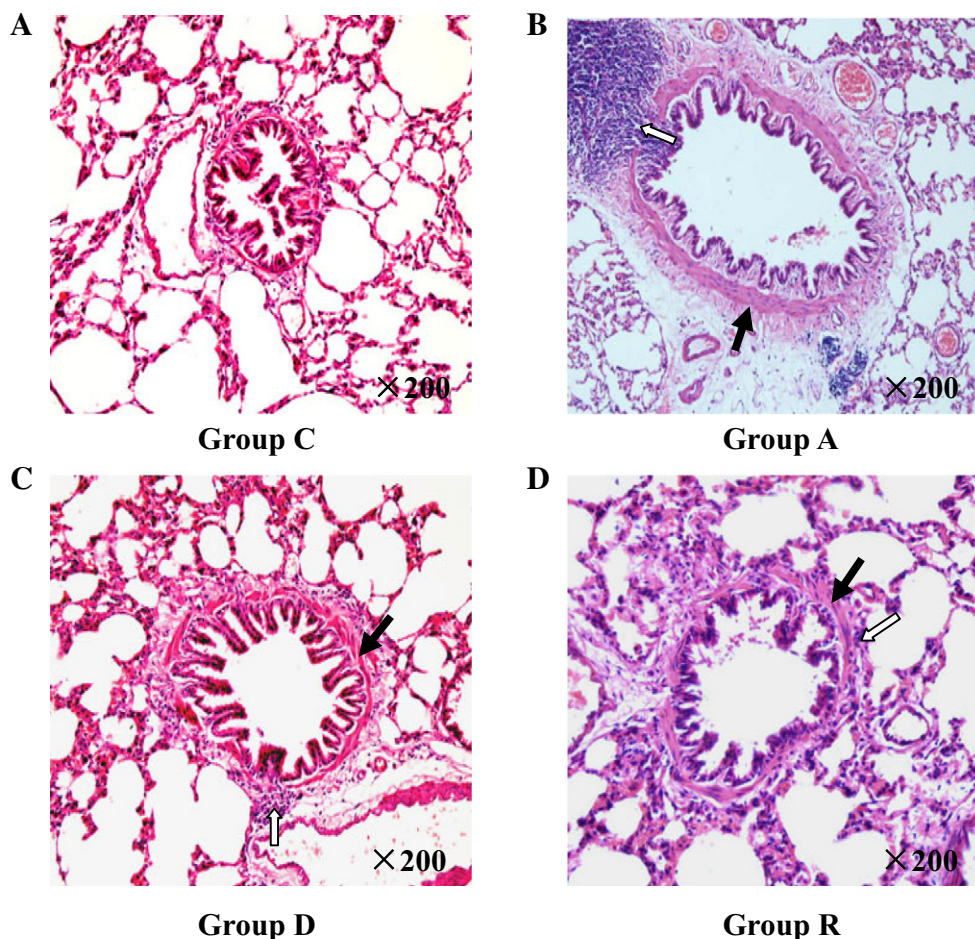


Fig. 1. Effects of RXM and dexamethasone on microstructure in lung tissue. Rats were randomized into asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D). Rats in Groups A, R and D were sensitized by ovalbumin/ $\text{Al}(\text{OH})_3$ mixture and challenged with ovalbumin. RXM (30 mg/kg) and dexamethasone (0.5 mg/kg) were given 0.5 h before the airway challenge initiation in Groups R and D, respectively; rats in Group C were sensitized and challenged with normal saline. After the 8-week challenge, the hematoxylin and eosin stained lung sections were examined under light microscope. Almost normal lung tissue was presented in Group C (A). In Group A, numerous inflammatory cells infiltration (white arrow), submucosal edema, airway smooth muscle hypertrophy and proliferation (black arrow) were observed (B). In Groups D (C) and R (D), there were less amounts of inflammatory cells, bronchial wall and smooth muscle layer are mild thickened.

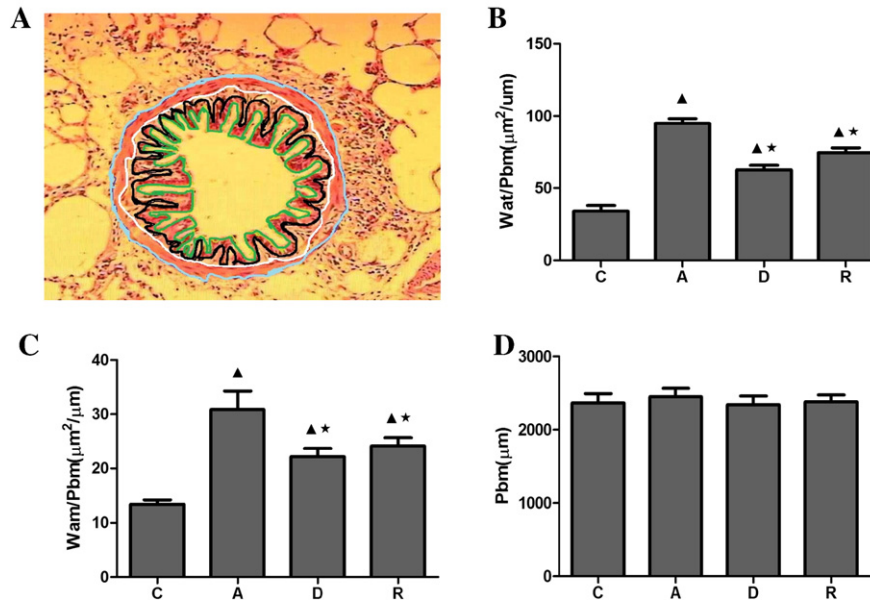


Fig. 2. Effects of RXM and dexamethasone on airway remodeling. Rats were randomized into asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D). Rats in Groups A, R and D were sensitized by ovalbumin/Al(OH)₃ mixture and challenged with ovalbumin. RXM (30 mg/kg) and dexamethasone (0.5 mg/kg) were given 0.5 h before the airway challenge initiation in Groups R and D, respectively; rats in Group C were sensitized and challenged with normal saline. After the 8-week challenge, bronchial smooth muscle cell layer was collected, Wat (the area between blue line and green line), Wam (the area between blue line and white line) and Pbm (the black line) were measured by Image-Pro Plus software 6.0 (A). Wat/Pbm (B) and Wam/Pbm (C) in Group A were significantly larger than those in Group C. As compared to Group A, Wat/Pbm (B) and Wam/Pbm (C) values were remarkably decreased in both Groups D and R, which were still higher than those in Group C. No significant differences in Pbm were found among groups (D). Pbm, basement membrane perimeter; Wat, total wall area; Wam, smooth muscle wall area. Data are expressed as mean $\pm$ SD. [▲] $P < 0.05$ vs. Group C; ^{*} $P < 0.05$ vs. Group A.

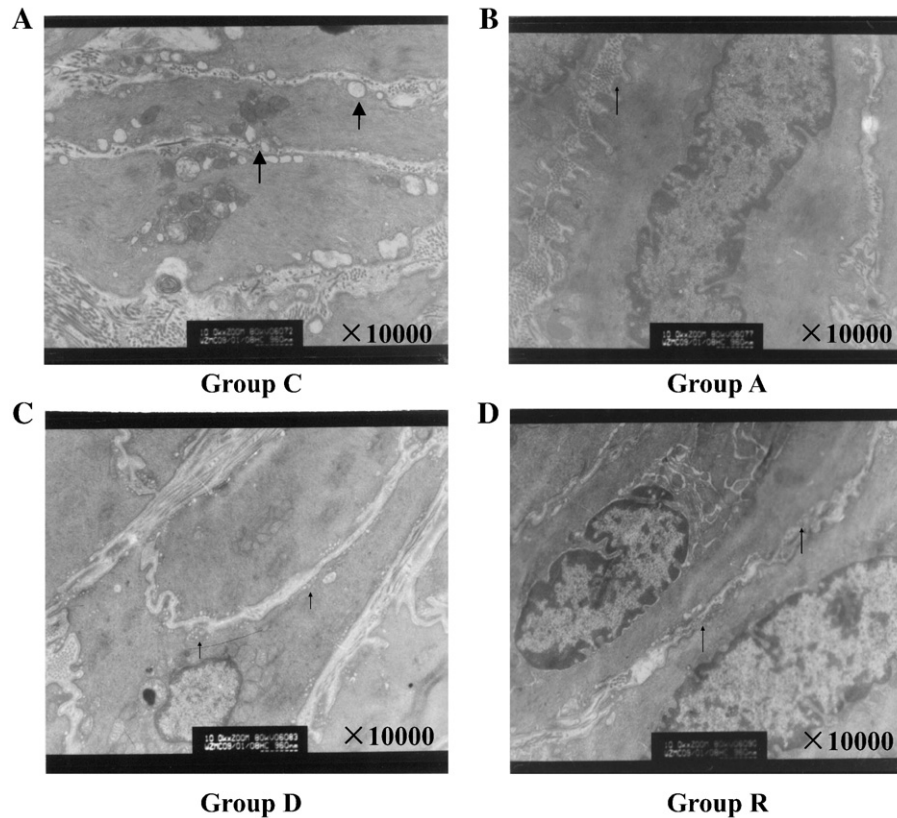


Fig. 3. Effects of RXM and dexamethasone on caveolae quantity in ASMCs. Rats were randomized into asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D). Rats in Groups A, R and D were sensitized by ovalbumin/Al(OH)₃ mixture and challenged with ovalbumin. RXM (30 mg/kg) and dexamethasone (0.5 mg/kg) were given 0.5 h before the airway challenge initiation in Groups R and D, respectively; rats in Group C were sensitized and challenged with normal saline. After the 8-week challenge, caveolae in ASMCs were observed under transmission electron microscopy. There were abundant caveolae (black arrow) with multiple shapes in ASMCs in Group C (A). In Group A, hypertrophy of ASMCs was represented with scarce caveolae (B). There are relatively abundant caveolae in ASMCs in Group D, but the amounts were less than in Group C (C). In Group R, more caveolae in ASMCs than in Group A were found, which were less than those in Groups C and D (D). ASMCs, airway smooth muscle cells.

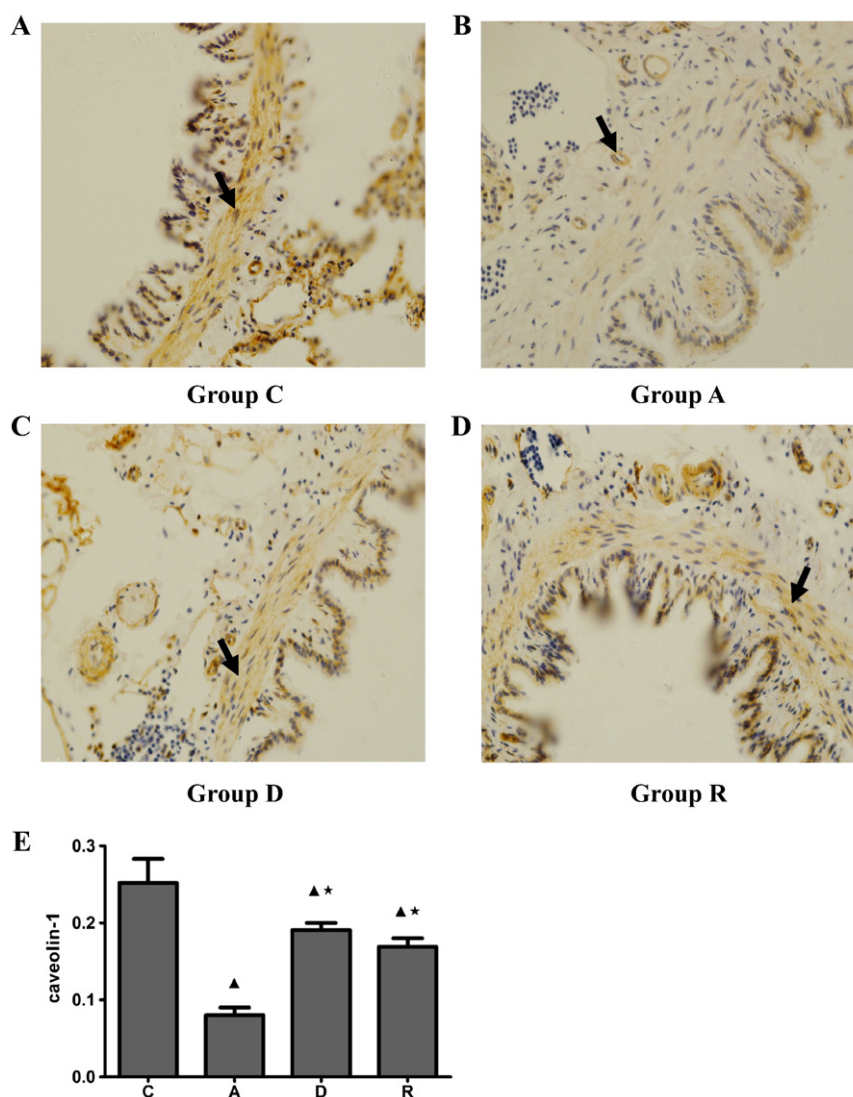


Fig. 4. Effects of RXM and dexamethasone on caveolin-1 expression by immunohistochemical assays. Rats were randomized into asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D). Rats in Groups A, R and D were sensitized by ovalbumin/Al(OH)₃ mixture and challenged with ovalbumin. RXM (30 mg/kg) and dexamethasone (0.5 mg/kg) were given 0.5 h before the airway challenge initiation in Groups R and D, respectively; rats in Group C were sensitized and challenged with normal saline. After the 8-week challenge, the expression of caveolin-1 in lung tissue was measured by immunohistochemical assays and the staining intensity was assessed quantitatively. Caveolin-1 stained in yellow mainly located in cytomembrane and cytoplasm (black arrow). The relative expression of caveolin-1 in Groups A, D and R was lower than that in Group C, and dexamethasone and RXM increased the expression of caveolin-1 as compared to Group A (E). Data are expressed as mean ± SD. ▲*P* < 0.05 vs. Group C, **P* < 0.05 vs. Group A.

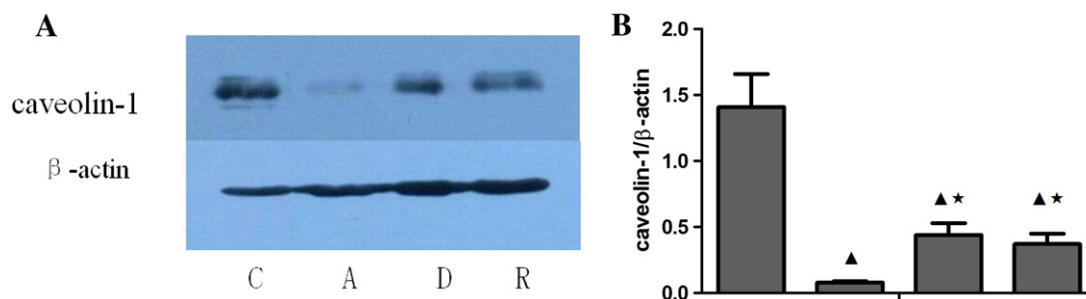


Fig. 5. Effects of RXM and dexamethasone on caveolin-1 expression by western blot. Rats were randomized into asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D). Rats in Groups A, R and D were sensitized by ovalbumin/Al(OH)₃ mixture and challenged with ovalbumin. RXM (30 mg/kg) and dexamethasone (0.5 mg/kg) were given 0.5 h before the airway challenge initiation in Groups R and D, respectively; rats in Group C were sensitized and challenged with normal saline. After the 8-week challenge, the expression of caveolin-1 in ASMCs was measured by western blot. The content of caveolin-1 in Group A was lower than that in Group C, and pretreatment with dexamethasone and RXM upregulated the expression of caveolin-1 (A, B). ASMCs, airway smooth muscle cells. Data are expressed as mean ± SD. ▲*P* < 0.05 vs. Group C, **P* < 0.05 vs. Group A.

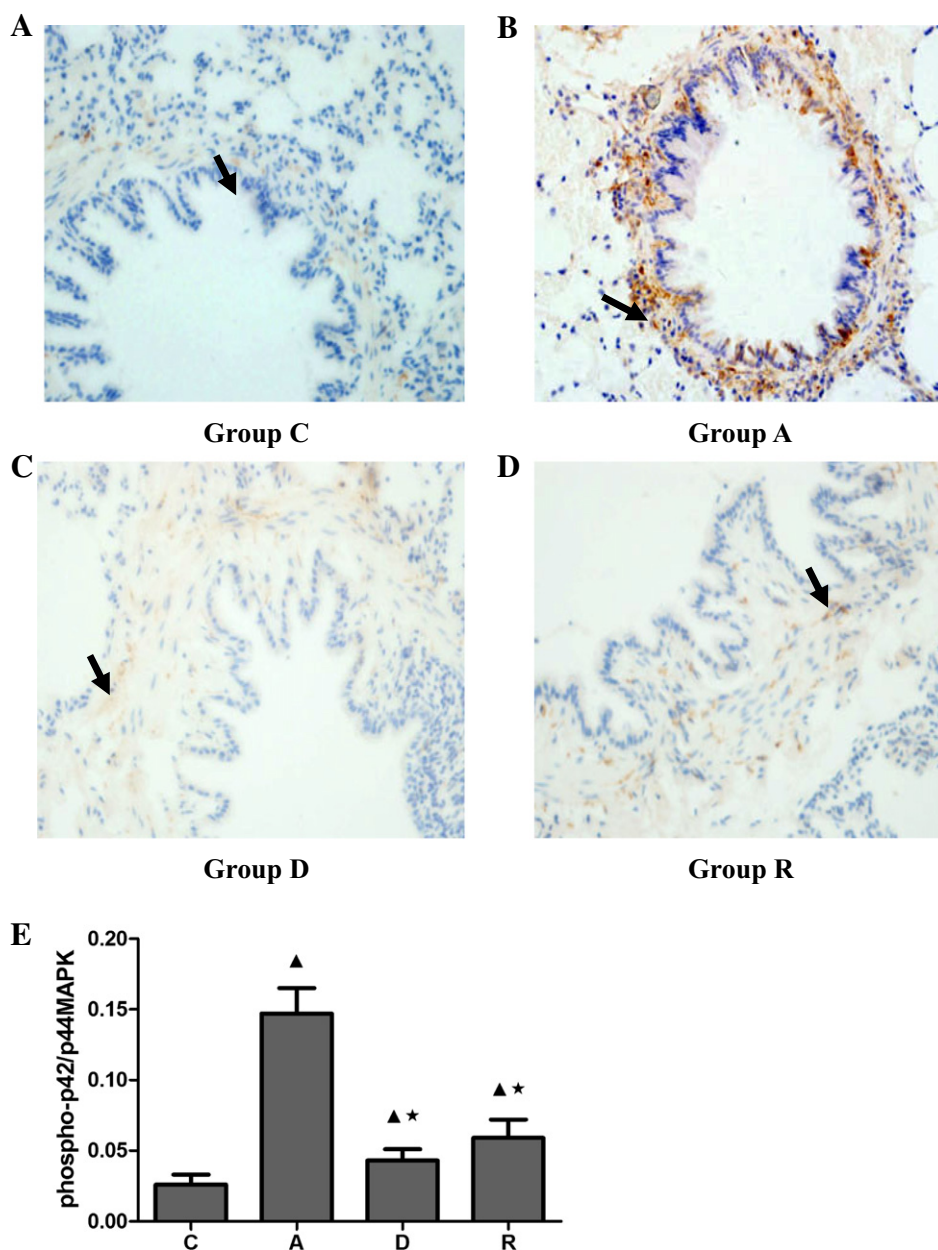


Fig. 6. Effects of RXM and dexamethasone on phospho-p42/p44MAPK expression by immunohistochemical assays. Rats were randomized into asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D). Rats in Groups A, R and D were sensitized by ovalbumin/Al(OH)₃ mixture and challenged with ovalbumin. RXM (30 mg/kg) and dexamethasone (0.5 mg/kg) were given 0.5 h before the airway challenge initiation in Groups R and D, respectively; rats in Group C were sensitized and challenged with normal saline. After the 8-week challenge, the expression of phospho-p42/p44MAPK in lung tissue was measured by immunohistochemical assays and the staining intensity was assessed quantitatively. phospho-p42/p44MAPK stained in yellow mainly located in cytoplasm (black arrow). The expression of phospho-p42/p44MAPK in Groups A, D and R was upregulated as compared to Group C, and dexamethasone and RXM decreased the expression of phospho-p42/p44MAPK as compared to Group A (E). ASMCs, airway smooth muscle cells. Data are expressed as mean ± SD. ▲*P* < 0.05 vs. Group C, **P* < 0.05 vs. Group A.

were dehydrated in acetone and embedded in Epon–Araldite resin. Ultrathin (50–100 nm) sections of these samples were stained with uranyl acetate and lead citrate. The caveolae in the airway smooth muscle cells (ASMCs) were observed by TEM (HF-2000, Hitachi, Japan).

2.4. Immunohistochemical analysis of caveolin-1 and phospho-p42/p44MAPK

4 μm-thick samples were deparaffinized in xylene and rehydrated in a graded series of ethanol. Antigen retrieval was achieved by heating

the slides in 10 mM sodium citrate (pH 6.0) followed by treatment with 3% H₂O₂. After blocking the antigen, sections were incubated with primary antibodies for caveolin-1 (diluted 1:75, Abcam Biotechnology, UK) or phospho-p42/p44MAPK (diluted 1:50, Cell Signaling Technology Inc., USA) at 4 °C overnight respectively. Slides were rinsed with PBS and incubated with secondary antibody for 30 min at 37 °C. Color was developed using DAB and counterstained with hematoxylin. The staining intensity of both caveolin-1 and phospho-p42/p44MAPK was assessed quantitatively under light microscopy by Image-Pro Plus (IPP) 6.0 (Media Cybernetics, USA) and expressed as integrated absorbency.

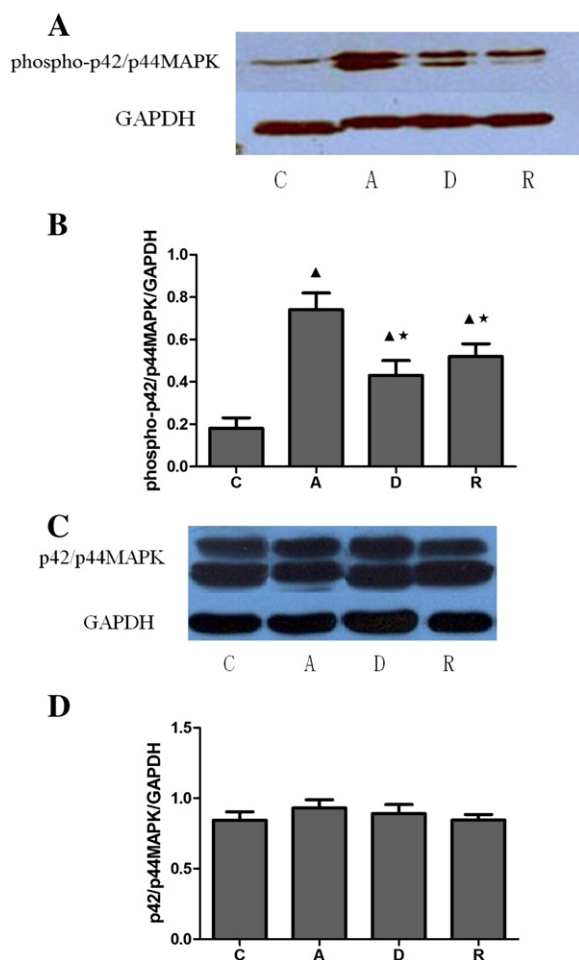


Fig. 7. Effects of RXM and dexamethasone on phospho-p42/p44MAPK expression by western blot. Rats were randomized into asthma group (Group A), control group (Group C), RXM-treated group (Group R) and dexamethasone-treated group (Group D). Rats in Groups A, R and D were sensitized by ovalbumin/Al(OH)₃ mixture and challenged with ovalbumin. RXM (30 mg/kg) and dexamethasone (0.5 mg/kg) were given 0.5 h before the airway challenge initiation in Groups R and D, respectively; rats in Group C were sensitized and challenged with normal saline. After the 8-week challenge, the expressions of phospho-p42/p44MAPK and total p42/p44MAPK in ASMCs were measured by western blot. The content of phospho-p42/p44MAPK protein in Group A was higher than that in Group C, the pretreatment with dexamethasone and RXM decreased the expression of phospho-p42/p44MAPK (A, B). No significant changes of total p42/p44MAPK were found among groups (C, D). ASMCs, airway smooth muscle cells. Data are expressed as mean $\pm$ SD. [▲] $P < 0.05$ vs. Group C, ^{*} $P < 0.05$ vs. Group A.

2.5. Western blot analysis of caveolin-1, phospho-p42/p44MAPK and total p42/p44MAPK

50 μ g protein samples of bronchial smooth muscle cell layer of each group were resolved by 12% sodium dodecyl sulfate-polyacrylamide gel. Then caveolin-1 and phospho-p42/p44MAPK were detected with caveolin-1 antibody (1:1000 dilution, Abcam Biotechnology, UK), phospho-p42/p44MAPK antibody (1:500 dilution, Cell Signaling Technology Inc., USA) and total p42/p44MAPK antibody (1:1000 dilution, Cell Signaling Technology Inc., USA), respectively. Blots were then incubated with horseradish peroxidase-linked immunoglobulin G secondary antibodies (Santa Cruz Biotechnology Inc., USA) for 1 h. Expression of β -actin or GAPDH was detected as an internal control and the relative density values for caveolin-1, phospho-p42/p44MAPK and total p42/p44MAPK were determined by densitometric analysis using Gel-pro software.

2.6. Statistical analysis

During the experimental protocol, one rat died in Group D and two rats in Group A. Thus data of randomized 6 rats were selected in each group for further statistical analysis. Results were expressed as means $\pm$ standard deviation ($\bar{x} \pm s$). Normal distribution data were analyzed for statistical significance by the method one-way ANOVA. Non-parametrical Kruskal–Wallis H statistics was used for data with abnormal distribution. Linear regression analysis was performed to determine correlation between different variables. P values < 0.05 were considered significant. Data were analyzed using SPSS 15.0 software.

3. Results

3.1. Effects of RXM and dexamethasone on microstructure in lung tissue

The structure of lung tissue in Group C including bronchial lumen and epithelial cells were intact; no abundant inflammatory cells infiltration or obvious thickening bronchial smooth muscle was observed (Fig. 1A). In Group A, numerous inflammatory cells mainly of eosinophils, neutrophils and lymphocytes infiltration in bronchial wall, interstitial tissue, alveoli and pulmonary capillaries were found (Fig. 1B). In addition, submucosal edema, increased mucosal fold, airway epithelium exfoliation, mucus plug, airway smooth muscle hypertrophy and proliferation, thickened bronchial wall, luminal stenosis, thickened and irregular basement membrane were observed (Fig. 1B). In Groups D (Fig. 1C) and R (Fig. 1D), there were less infiltrated inflammatory cells, mildly thickened bronchial wall and smooth muscle layer, other microstructural changes including airway smooth muscle hypertrophy and proliferation were also ameliorated. Wat/Pbm in Groups A, D and R was significantly increased as compared to Group C, and its value in both Groups D and R was lower than that in Group A ($P < 0.05$, Fig. 2B). Similarly, as compared to Group C, Wam/Pbm values were significantly increased in Groups A, D and R, and Wam/Pbm in both Groups D and R were lower than those in Group A ($P < 0.05$, Fig. 2C). Differences between Groups D and R in both Wat/Pbm and Wam/Pbm were not significant ($P > 0.05$). No significant differences in Pbm were found among groups ($P > 0.05$) (Fig. 2D).

3.2. Effects of RXM and dexamethasone on caveolae quantity in ASMCs

Abundant caveolae with multiple shapes in ASMCs in Group C (Fig. 3A) and scarce caveolae in Group A were presented (Fig. 3B). The amounts of caveolae in ASMCs in both Groups D (Fig. 3C) and R (Fig. 3D) were lower than those in Group C, and more caveolae were presented in ASMCs in Group D as compared to Group R.

3.3. Effects of RXM and dexamethasone on caveolin-1 expression in ASMCs

Immunohistochemical assay showed that caveolin-1 mainly expressed in cytomembrane and cytoplasm in ASMCs (Fig. 4A–D). As measured by both immunohistochemical assay (Fig. 4E) and western blot (Fig. 5A, B), the expressions of caveolin-1 in Groups A, D and R were lower than those in Group C, and the pretreatment with dexamethasone and RXM increased the expression of caveolin-1 as compared to Group A ($P < 0.05$). There were no significant differences between Groups D and R ($P > 0.05$).

3.4. Effects of RXM and dexamethasone on phospho-p42/p44MAPK expression in ASMCs

Immunohistochemical assays demonstrated cytoplasmic expression of phospho-p42/p44MAPK in ASMCs (Fig. 6A–D). As demonstrated by both immunohistochemical assay (Fig. 6E) and western blot (Fig. 7A, B), the expressions of phospho-p42/p44MAPK in Groups A, D and R were significantly higher than those in Group C, which could be

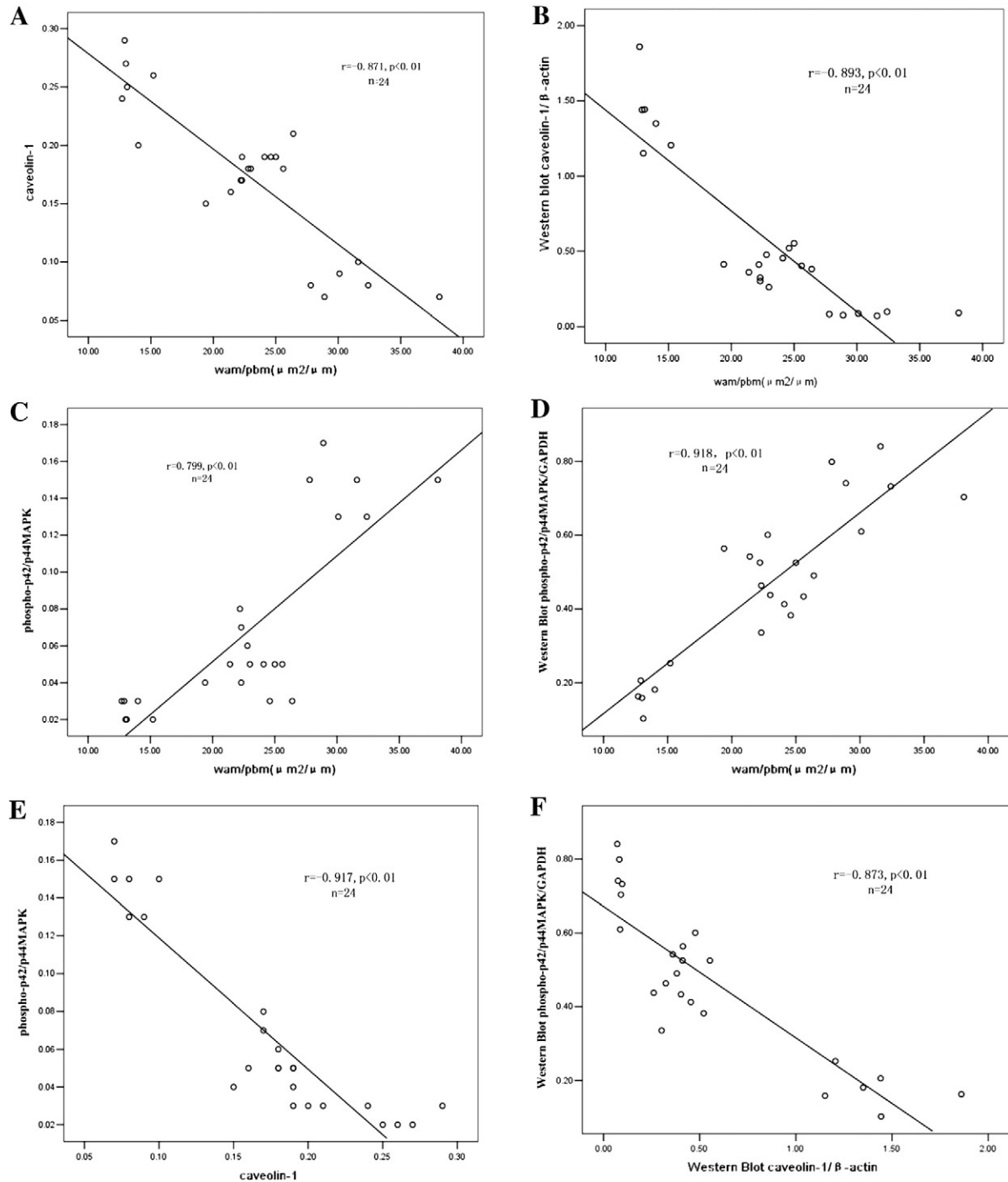


Fig. 8. The correlative analysis between different parameters. Wam/Pbm correlated negatively to the expression of caveolin-1 by both immunohistochemical assay ($r = -0.871$, $n = 24$, A) and western blot ($r = -0.893$, $n = 24$, B); Wam/Pbm correlated positively to phospho-p42/p44MAPK by both immunohistochemical assay ($r = 0.799$, $n = 24$, C) and western blot ($r = 0.918$, $n = 24$, D). The expression of caveolin-1 correlated negatively to phospho-p42/p44MAPK by both immunohistochemical assay was found ($r = -0.917$, $n = 24$, E) and western blot ($r = -0.873$, $n = 24$, F). Pbm, basement membrane perimeter; Wat, total wall area; Wam, smooth muscle wall area.

downregulated by both dexamethasone and RXM ($P < 0.05$). No differences between Groups D and R were found ($P > 0.05$). Total p42/p44MAPK expression did not differ among these groups (Fig. 7C, D).

3.5. The correlation analysis among different parameters

Wam/Pbm correlated negatively to the expression of caveolin-1 by both immunohistochemical assay ($r = -0.871$, $P < 0.05$, $n = 24$, Fig. 8A) and western blot ($r = -0.893$, $P < 0.05$, $n = 24$, Fig. 8B), and it correlated positively to the phospho-p42/p44MAPK expression by

immunohistochemical assay ($r = 0.799$, $P < 0.05$, $n = 24$, Fig. 8C) and western blot ($r = 0.918$, $P < 0.05$, $n = 24$, Fig. 8D). A negative correlation between the expression of caveolin-1 and phospho-p42/p44MAPK by both immunohistochemistry ($r = -0.917$, $P < 0.05$, $n = 24$, Fig. 8E) and western blot ($r = -0.873$, $P < 0.05$, $n = 24$, Fig. 8F) was found.

4. Discussion

In this study, the obvious pathological changes of chronic asthmatic rats, including structural damages and thickening bronchial wall and

bronchial smooth muscle cell layer, demonstrated that the airway remodeling model was successfully established. The major findings of our present study were that RXM could suppress the airway remodeling process and modulate the expression of caveolin-1 and phospho-p42/p44MAPK in chronically asthmatic rats, and its effects were similar to the traditional steroid dexamethasone.

Phenotype plasticity of ASMCs is associated with changes of cellular contractility, proliferation, migration and secretion of inflammatory mediators [17,18], which contributes to the asthmatic airway remodeling [19]. Caveolae is very important in the phenotypic transformation of smooth muscle cells, and the number of caveolae was reduced when the arterial smooth muscle cells converted from a contractile to a synthetic phenotype [20,21]. Caveolin-1, one of the main structural and functional proteins of caveolae of ASMCs [22], appears to play a decisive role in the proliferation and migration of smooth muscle cells [23]. Caveolin-1 siRNA could result in the proliferation of ASMCs in mitogen deficient conditions [11], while caveolin-1 knockout would lead to enhanced remodeling with increased proliferation markers and collagen [24]. These studies indicate that caveolin-1 may negatively regulate ASMCs proliferation. In our present study, TEM showed that there were significant proliferation of ASMCs and fewer caveolae with structure destroyed in ASMCs in asthmatic rats. Additionally, the significantly decreased expression of caveolin-1 presented in chronic asthma model, which also negatively correlated with the thickness of bronchial smooth muscle cell layer. These findings were consistent with the view that caveolin-1 plays a key role in maintaining quiescence of ASMCs and suppressing asthmatic airway remodeling.

p42/p44MAPK belongs to the MAPK family and is located downstream of the cascade of Ras/Raf/MEK kinase. When phosphorylated, p42/p44MAPK is activated and can translocate into the nucleus and activate transcription factors required for cell proliferation. Antisense oligodeoxynucleotides directed at p42/p44MAPK mRNAs reduced p42/p44MAPK protein and attenuated human the proliferation of ASMCs [25]. In our study, although antigen challenge had no effect on total p42/p44MAPK content, the expression of phospho-p42/p44MAPK was increased, which positively correlated with the thickness of the bronchial smooth muscle cell layer. Caveolin-1 also has been demonstrated to compartmentalize and integrate a wide range of signal transduction processes as well as to transport small molecules [26]. Research showed that caveolin-1 expression inhibited signal transduction from the p42/p44MAPK cascade both in vitro and in vivo, modulating cellular proliferation of ASMCs, a major aspect of diseases such as asthma [11,27]. The previous studies together with our study indicated the possible associations between caveolin-1 and p42/p44MAPK activation in airway remodeling in asthmatic status.

Dexamethasone has been proven to inhibit ASMCs proliferation induced by various stimuli, such as thrombin, epidermal growth factor, basic fibroblast growth factor and fetal calf serum [28]. And pretreatment with dexamethasone before OVA-challenge in sensitized mice can partially ameliorate the structural changes associated with airway remodeling [29]. Additionally, pretreatment with dexamethasone induces caveolin-1 at both protein and mRNA levels in a time- and dose-dependent manner in pharmacologically relevant concentrations in cultured aortic endothelial cells [30]. Therefore, dexamethasone has been set as an effective control. RXM expresses immunomodulatory effect that is distinct from their anti-infection properties [31]. Although not inhibiting bronchoconstriction and leukotriene overproduction, RXM did represent an anti-inflammatory effect associated with eosinophilic infiltration in patients with aspirin-intolerant asthma [32]. Moreover, RXM could suppress goblet cell hyperplasia and cytokine secretion by regulating the activation of MAPK/NF- κ B pathway, leading to lower airway hyperresponsiveness and less inflammatory cells infiltration in the lung tissue of mice with ovalbumin-induced allergic asthma [16]. But the effects of RXM on airway remodeling and caveolin-1 in chronic asthma model are still unknown. Our study demonstrated that pretreatment with RXM can reduce asthmatic airway remodeling, which was

evidenced by the decrease in Wat/Pbm and Wam/Pbm, and also RXM pretreatment could increase the caveolin-1 expression and meanwhile lower the phospho-p42/p44MAPK expression. Our study suggested that RXM could be considered as a complementary medication in the therapy of chronic asthma especially for those intolerant to steroids therapy. One of the limitations of our study is that, because the commercial injection of RXM is not available, it was given intragastrically, while its counter-treatment dexamethasone was intraperitoneally administered. Secondly, knockout or siRNA methods were not used to determine the role of caveolin-1 in attenuating airway remodeling of RXM or dexamethasone.

In conclusion, RXM pretreatment can present similar effects as dexamethasone in suppressing asthmatic airway remodeling, and increasing caveolin-1 expression and inhibiting the activation of p42/p44MAPK pathway in chronic asthmatic rats.

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