



Azithromycin ameliorates OVA-induced airway remodeling in Balb/c mice via suppression of epithelial-to-mesenchymal transition

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ARTICLE INFO

Keywords:

Azithromycin
Airway remodeling
Epithelial-to-mesenchymal transition (EMT)
TGF- β 1/RACK1
Balb/c mice

ABSTRACT

Azithromycin is a potent agent that prevents airway remodeling. In this study, we hypothesized that azithromycin (35 mg/kg orally) alleviated airway remodeling through suppression of epithelial-to-mesenchymal transition (EMT) via downregulation of transforming growth factor-beta 1 (TGF- β 1)/receptor for activated C-kinase1 (RACK1)/snail in mice. An ovalbumin (OVA)-induced Balb/c mice airway allergic inflammatory model was used. Airway inflammation and remodeling were evaluated with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Masson staining. E-cadherin and N-cadherin (molecular markers of EMT) were analyzed by immunofluorescence, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), and western blotting; α -smooth muscle actin (α -SMA) was evaluated using immunohistochemistry (IHC), qRT-PCR, and western blotting; and expression of TGF- β 1/RACK1/Snail in lungs was measured by qRT-PCR and western blotting. Our data showed that azithromycin significantly reduced inflammation score, peribronchial smooth muscle layer thickness, goblet cell metaplasia, and deposition of collagen fibers ($P < 0.05$), and effectively suppressed airway EMT (upregulated E-cadherin level, and downregulated N-cadherin and α -SMA levels) compared with the OVA group ($P < 0.05$). Moreover, the increasing mRNA and protein expressions of TGF- β 1 and RACK1 and mRNA level of Snail in lung tissue were all significantly decreased in azithromycin-treated mice ($P < 0.05$). Taken together, our results suggest that azithromycin has the greatest effects on reducing airway remodeling by inhibiting TGF- β 1/RACK1/Snail signal and improving the EMT in airway epithelium.

1. Introduction

Azithromycin, a macrolide antibiotic, exhibits promising effects on exacerbations of asthma [1]. Our previous studies have indicated that azithromycin (25 mg/kg) has significant protective effects against allergic airway inflammation and airway remodeling by inhibiting transforming growth factor-beta 1 (TGF- β 1) expression [2] and inhibiting epithelial cell apoptosis in rat lungs [3]. Interestingly, azithromycin has been reported to inhibit TGF β 1-induced airway epithelial-mesenchymal transition (EMT) by suppressing Smad3 production in airway epithelial cells [4], implying that azithromycin might be used to treat asthma through ameliorating airway EMT. However, the exact cellular mechanism of azithromycin on airway EMT in asthma has never been investigated.

Airway remodeling is the key pathologic feature of asthma and is associated with the persistence of asthma symptoms and poor clinical outcomes [5]. The pathogenesis of asthmatic airway remodeling is generally believed to be associated with epithelial-mesenchymal

transition [6]. Epithelial-mesenchymal transition is a dynamic process in which epithelial cells gradually lose their epithelial characteristics and acquire mesenchymal features. Currently, increasing evidence demonstrated that TGF- β 1 is the initiation of EMT, and the development of EMT is generally accompanied with the loss of epithelial markers such as E-cadherin and the expression of mesenchymal markers such as N-cadherin and α -smooth muscle actin (α -SMA) [7]. In recent years, a number of studies have highlighted a critical role of snail on E-cadherin regulation [8] via binding E-cadherin promoters (E-boxes) [9], especially in asthma [10]. Moreover, previous studies have demonstrated that Snail was directly activated by the TGF- β 1/Smad signaling pathway [11,12] or indirectly mediated by receptor for activated C-kinase1 (RACK1) [13], which is the downstream target gene of TGF- β 1 in primary hepatic stellate cell (HSCs) [14]. Notably, azithromycin was also documented to recover the reduction of E-cadherin level induced by TNF- α in gingival epithelial cells [15].

Based on these literature evidences, we designed our study to investigate whether azithromycin possessed its antifibrotic effect through

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<https://doi.org/10.1016/j.intimp.2018.03.016>

Received 11 December 2017; Received in revised form 15 March 2018; Accepted 16 March 2018
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suppressing EMT by blocking activation of the TGF- β 1 pathway in OVA-induced Balb/c mice.

2. Materials and methods

2.1. Animals and ethics statement

Twenty-four male Balb/c mice, aged 5 to 6 weeks and weighing 20 ± 2 g, were obtained from Da-Shuo Biological Technology Co., Ltd. (Chengdu, China). The mice were acclimated for 1 week under standard conditions of ambient temperature ($22^\circ\text{C} \pm 1^\circ\text{C}$), humidity ($60\% \pm 10\%$), and with a 12-h light/dark cycle (lights on at 8:00 am). All experimental procedures were conducted in accordance with the guidelines of the Experimental Research Institute of Sichuan University in agreement with the guidelines of the Canadian Council on Animal Care (Permit Number: 2003-149). Animal ethics approval has been obtained from West China School of Basic Medical Sciences and Forensic Medicine.

2.2. Experimental groups

All mice were randomly divided into four groups ($n = 6$) including the control group (saline challenge + saline treatment), the OVA group (OVA challenge + saline treatment), the dexamethasone (DEX) group (OVA challenge + 0.7 mg/kg DEX treatment), and the azithromycin (AZM) group (OVA challenge + 35 mg/kg AZM treatment, corresponding to human clinical equivalent dosage of 250 mg/d).

2.3. OVA-induced mice airway allergic inflammatory model and treatment

Balb/c mice were sensitized by an intraperitoneal injection of 20 μg of OVA (Sigma-Aldrich, USA) in 0.2 mL saline and 20 mg aluminum hydroxide (Aldrich, USA) in 0.2 mL of saline on days 0 and 14. From day 21, mice were given aerosol challenges with 1%OVA for 60 min using an ultrasonic nebulizer (NE-U12; Omron Co., Tokyo, Japan) once daily. The control mice were challenged with saline instead of 1%OVA aerosol in a similar manner. From day 21, OVA-sensitized mice were administrated with 0.2 mL of saline containing DEX (0.7 mg/kg) or AZM (35 mg/kg) orally 1 h prior to the 1%OVA challenge, whereas the control mice were treated in the same way with normal saline. On day 28, all mice were euthanized and decapitated. The inferior lobes of the right lungs from 6 animals in each group were rapidly removed, dissected, and stored at -80°C . The left lung lobes were immediately fixed in 10% (v/v) neutral-buffered formalin for 24 h at 4°C and embedded in paraffin. The schematic diagram of the treatment schedule is shown in Fig. 1.

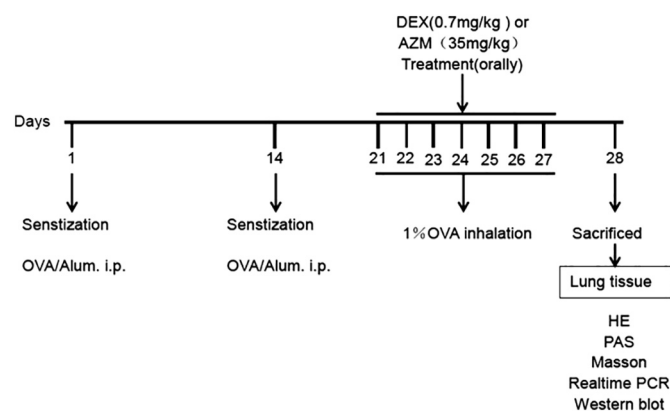


Fig. 1. Mouse model of airway allergic inflammatory and treatment with azithromycin.

2.4. Lung tissue histopathology

The tissues were sectioned at 4 μm thickness and stained with hematoxylin and eosin (HE) (Sigma, St. Louis, MO), periodic acid–Schiff (PAS) solution (IMEB, San Marcos, CA), and Masson trichrome staining to assess the inflammation, epithelial injury, and degree of collagen deposition, respectively. The airway wall thickness (Wat) and the bronchial smooth muscle thickness (Wam) were quantified by Image-Pro® Plus 6.0 software (Media Cybernetics, Bethesda, MD) by adjusting to basement membrane length (μm), and the inflammatory degree was scored by two independent observers blinded to the experiment. The detail methods refer to our previous publication [3]. An increment of 0.5 was given if the inflammation scores were in between two grades, and the total inflammation score was calculated by the addition of both peribronchial and perivascular inflammation scores ($n = 10$ airways from 3 to 5 animals). The thickness of the epithelial layer and mucus accumulation in the epithelial layer were quantified using PAS-stained sections with Image-Pro® Plus 6.0 software by dividing the epithelial area (μm^2) or the PAS⁺ area in the epithelium (μm^2) by the length of the basement membrane (μm) around a bronchus. The bronchi (0.4–0.6 mm in diameter) in each animal were randomly chosen to quantify the fibrosis score. The degree of fibrosis was graded from 0 to 3 using Masson-stained sections, and the detail methods refer to our previous publication [16].

2.5. Immunohistochemistry (IHC)

Paraffin-embedded sections of lung tissue were assessed with IHC. After quenching with 3% H_2O_2 , the sections were incubated with anti- α -SMA antibody (1:2000, G13044, Servicebio, China) overnight at 4°C , followed by incubation with a secondary antibody (G1211, Servicebio, China) at room temperature for 50 min. Immunoreactivity was visualized with diaminobenzidine (DAB) (G1211, Servicebio, China). Brown staining was considered positive. Images were captured using an Olympus microscope (IX51; Olympus Corporation, Tokyo, Japan). The sections were evaluated by Image-Pro Plus 6.0 software, and the average of optical density (AOD) was quantified at $200\times$ magnification.

2.6. Immunofluorescence

Co-staining was performed on lung tissue sections. Antigen retrieval was performed in a pressure cooker using ethylenediamine tetraacetic acid (EDTA; pH 8.0) for 20 min in boiling water. Then, sections were incubated overnight at 4°C with primary antibodies raised against E-cadherin (GB11082; 1:200; Guge, China) and N-cadherin (GB11135; 1:400; Guge, China). Subsequently, CY3 conjugated goat anti-rabbit IgG antibody (GB25303; 1:300; Guge, China) was used as a secondary antibody and incubated with the sections for 50 min at room temperature. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, 1:200) for 10 min at room temperature. Images were captured using an Olympus microscope (IX51; Olympus Corporation, Tokyo, Japan).

2.7. mRNA and protein preparation

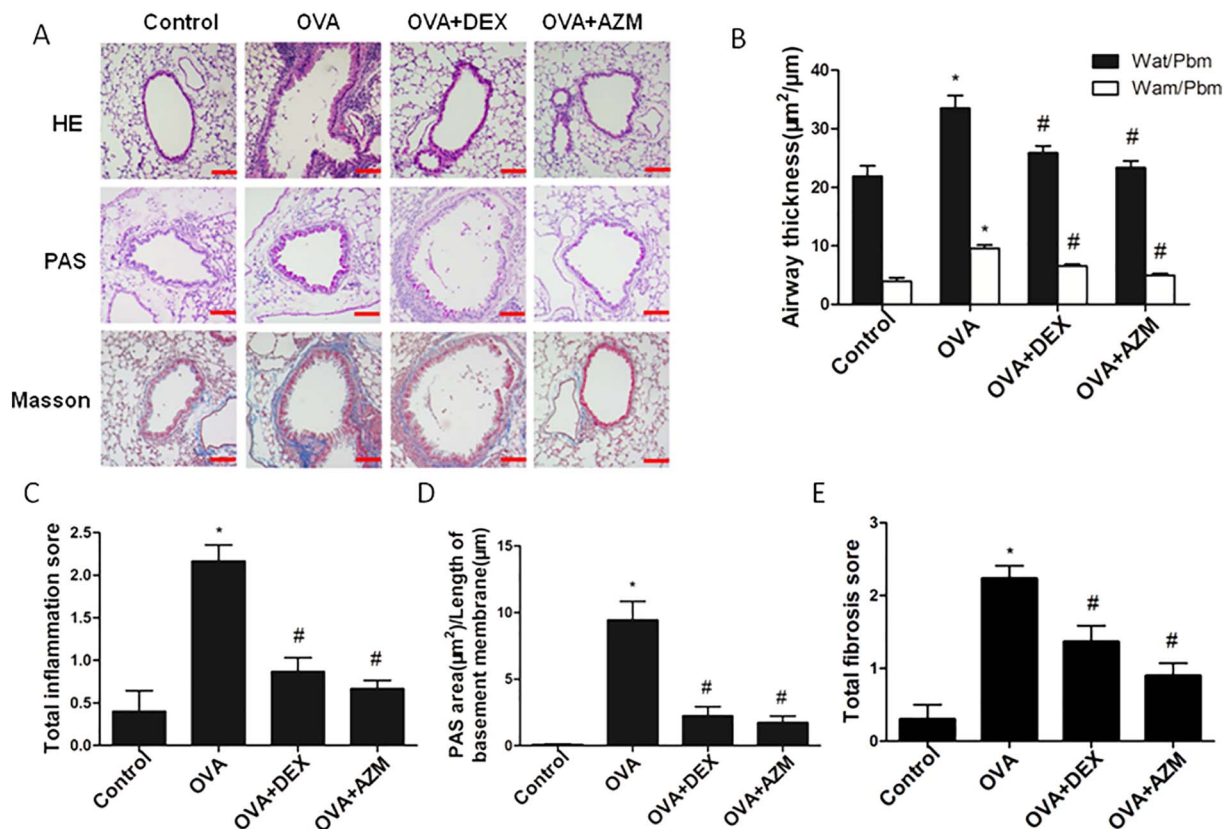
The total RNA and protein of lung were extracted by the Eastep®Super Total RNA Extraction Kit (Promega, China). Protein concentrations were determined using the BCA protein assay kit (Beyotime Institute of Biotechnology, China).

2.8. qRT-PCR

Total RNA (1 μg) was reverse-transcribed with a Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA). The CFX96™ Real-Time PCR Detection System (Bio-Rad, USA) was applied for the mRNA amplification of related genes using the following forward and reverse primers

Table 1
PCR primers.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Condition	PCR product length (bp)
GAPDH	GGTGTCTCTCGCACTTCA	GGGTGGTCCAGGGTTTCTTA	95 °C 30s, (95 °C 5 s, 65 °C 60 s, 72 °C 30s) for 39 cycles, melt curve.	186
E-cad	CAGGTCTCTCTCATGGCTTTGC	CTTCCGAAAAGAAGGCTGTCC	95 °C 30s, (95 °C 5 s, 65 °C, 60s) for 39 cycles, melt curve.	175
N-cad	AGCGCAGTCTTACCGAAGG	TCCGTGCTTTTCACTGAACCTT	95 °C 30s, (95 °C 5 s, 65 °C, 60s) for 39 cycles, melt curve.	101
α-SMA	CCCAACTGGGACCATGG	TACATGCGGGGACATTGAAG	95 °C 30s, (95 °C 5 s, 55 °C, 60s) for 39 cycles, melt curve.	170
Snail	CACACGCTGCTTGTGTCT	GGTCAGCAAAAGCACGGTT	95 °C 30s, (95 °C 5 s, 65 °C, 30s) for 39 cycles, melt curve.	133
TGF-β1	TCGCTGCTTTCATACTGAACCTT	GCCTTAGTTTGACAGGATCTG	95 °C 30s, (95 °C 5 s, 60 °C, 30s) for 39 cycles, melt curve.	133
RACK1	GGATCTCAATGAAGGCAAGC	TTGCTGCTGGTGTGATAAC	95 °C 30s, (95 °C 5 s, 60 °C, 30s) for 39 cycles, melt curve.	183

**Fig. 2.** Protective effects of azithromycin on OVA-induced lung inflammation and airway remodeling.

Histological examination of lung tissue was performed 24 h after the final OVA challenge. Lung tissues were fixed, sectioned at 4 μm thickness, and stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and Masson stain. (A) Representative HE (upper panel), PAS (middle panel), and Masson (lower panel) stained sections of lung of mice in the control group, OVA group, OVA + DEX group, and OVA + AZM group (magnification × 200). (B) Quantified results of airway wall thickness (Wat) and bronchial smooth muscle thickness (Wam) analyzed by Image-Pro® Plus 6.0 software. (C) Total inflammation score. (D) Degree of mucus accumulation was calculated by dividing PAS⁺ area (μm²) in the bronchus by the length (μm) of basement membrane. (E) Total fibrosis score. Values are presented as mean ± S.E.M. *P < 0.05, versus control group (n = 6). #P < 0.05, versus OVA group (n = 6).

(Table 1). The amplification conditions were as shown in Table 1. The mRNA levels of E-cad, N-cad, Snail, α-SMA, TGF-β1, and RACK1 were normalized by the expression of GAPDH. Experiments were performed in triplicate. All the data were analyzed using the $2^{-\Delta\Delta Ct}$ method ($\Delta Ct = Ct_{\text{Target gene}} - Ct_{\text{GAPDH}}$, $\Delta\Delta Ct = \Delta Ct_{\text{Exp}} - \Delta Ct_{\text{Control}}$).

2.9. Western blotting

Equal amounts of total protein were loaded onto 10% SDS-PAGE gels. Loaded proteins were separated at 100 V for 60 min and electrotransferred to PVDF membranes by the wet transfer method (200 mA, 90 min). The membranes were blocked in 5% fat-free milk at 4 °C overnight. Subsequently, the membranes were incubated with primary antibody at room temperature for 2 h, including anti-GAPDH (ZSGB-BIO, China) antibody (dilution 1:1000), anti-TGF-β1 (R&D, USA) antibody (dilution 1:500), anti-RACK1 (Santa Cruz Biotechnology, USA) antibody (dilution 1:100), anti-E-Cadherin Rabbit mAb (CST, USA)

antibody (dilution 1:1000), anti-N-Cadherin (abcam, UK) antibody (dilution 1:1000), anti-α-SMA (Proteintech, USA) antibody (dilution 1:500), and anti-Snail (Wanleibio, China) antibody (dilution 1:1000). After washing with TBST, the membranes were incubated with secondary goat anti-mouse IgG (ZB-2305, ZSGB-BIO, China) antibody (dilution 1:1000) at room temperature for 1 h, and were conjugated with horseradish peroxidase. Then, proteins were detected by chemiluminescence reagent (GE Healthcare). Protein band density was quantified using Bio-Rad Quantity One v4.62. The relative protein levels were normalized to GAPDH level.

2.10. Statistics

Statistical analysis was performed by one-way analysis of variance (ANOVA) with Bonferroni correction (GraphPad Prism version 5). All values were expressed as mean ± SEM, and P < 0.05 was considered to be statistically significant.

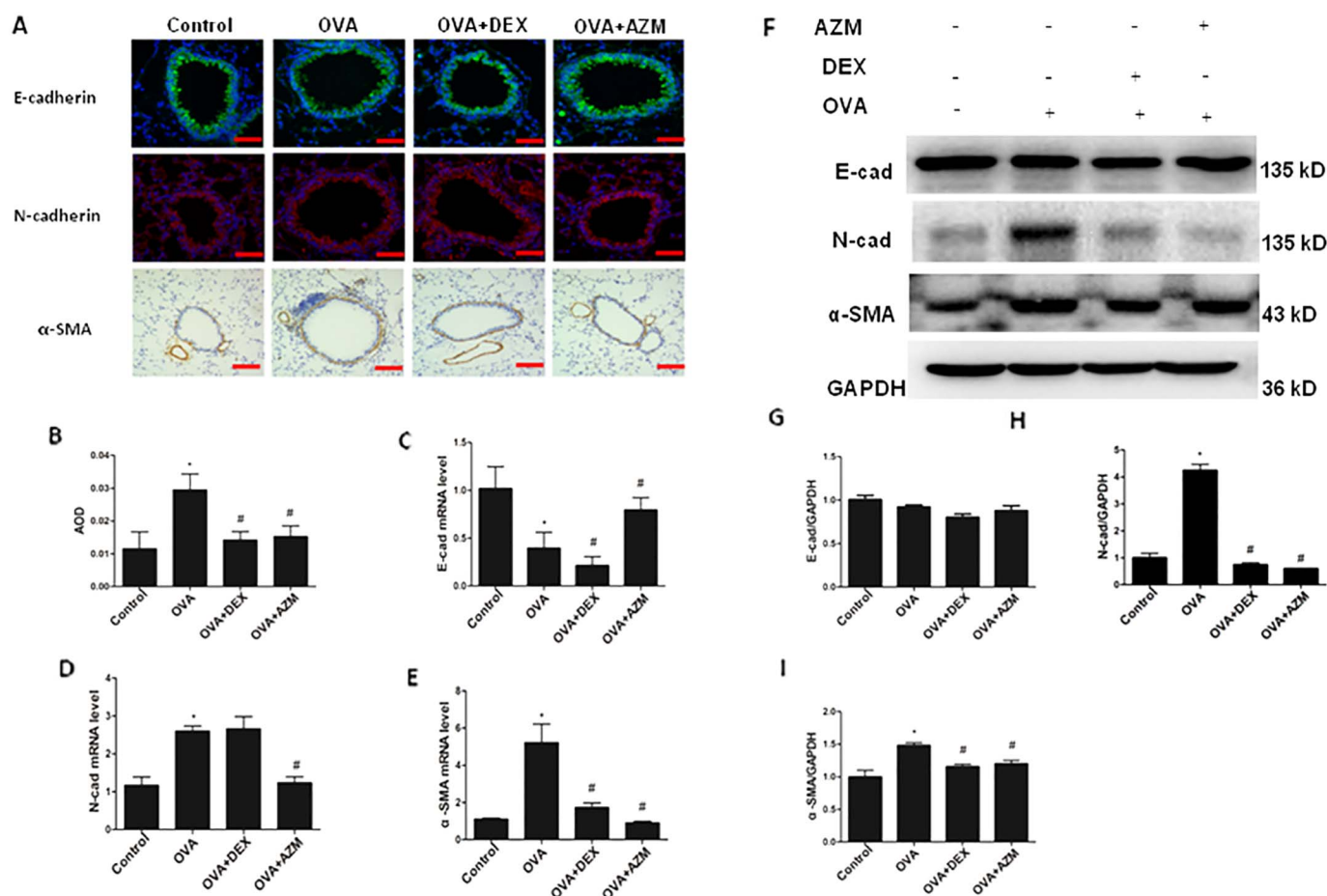


Fig. 3. Protective effects of azithromycin on OVA-induced airway epithelial-to-mesenchymal transition (EMT).

(A) Representative immunofluorescent (upper: E-cadherin; middle: N-cadherin; magnification $\times 400$) and immunohistochemistry (IHC) (lower: α -SMA; magnification $\times 200$) stained sections of lung of mice in the control group, OVA group, OVA + DEX group, and OVA + AZM group. (B) Quantified results of α -SMA. (C–E) The mRNA levels of E-cadherin, N-cadherin, and α -SMA were determined by quantitative real-time polymerase chain reaction (qRT-PCR). mRNA value = $2^{-\Delta\Delta Ct}$, $\Delta\Delta Ct = (Ct_{Target} - Ct_{GAPDH})$ of the treated mice. $(Ct_{Target} - Ct_{GAPDH})$ of the control mice. (F) The protein expressions of E-cadherin, N-cadherin, and α -SMA were analyzed by western blotting. GAPDH was used as an internal standard. (G–I) The relative levels of E-cadherin, N-cadherin, and α -SMA expressions in the lung. The results were expressed as the ratio of the investigated gene to GAPDH. The band intensity was analyzed by Bio-Rad Quantity One v4.62 software. Values are presented as mean $\pm$ S.E.M. * $P < 0.05$, versus control group ($n = 6$). # $P < 0.05$, versus OVA group ($n = 6$).

3. Results

3.1. Azithromycin alleviates OVA-induced airway inflammation and remodeling in mice

To confirm the potential effect of azithromycin in Balb/c mice, mice challenged with 1%OVA as described previously were treated with DEX (0.7 mg/kg) or AZM (35 mg/kg) (daily, intragastrically) for 1 week, and histopathologic analysis was performed on HE, PAS, and Masson stained lung sections. As shown in Fig. 2A, the 1%OVA challenge significantly induced peribronchial infiltration of inflammatory cells compared with the control mice. Conversely, treatment with AZM or DEX significantly ameliorated the inflammatory changes around the bronchus (Fig. 2A), which was confirmed by the inflammation score. Compared with control mice, inflammatory scores were significantly increased in the OVA-challenged mice ($P < 0.05$) (Fig. 2C), which as expected, were notably decreased by AZM or DEX treatment ($P < 0.05$) (Fig. 2C). Similarly, AZM or DEX intervention significantly reduced the damage to normal lung architecture and inhibited excessive collagen deposition due to OVA, as determined by HE, PAS, and Masson staining.

The thickness of the peribronchial smooth muscle layer (Fig. 2B), goblet cell metaplasia (mucus production) (Fig. 2D), and collagen deposition (Fig. 2E) were also significantly increased in the OVA-challenged mice ($P < 0.05$). However, AZM- and DEX-treated mice

demonstrated a significant reduction in thickness of the peribronchial smooth muscle layer (Fig. 2B), goblet cell metaplasia (mucus production) (Fig. 2D), and collagen deposition (Fig. 2E), when compared with the OVA-challenged mice ($P < 0.05$).

3.2. Azithromycin represses epithelial-to-mesenchymal transition in mice

To investigate whether EMT occurs in lung tissue from the OVA-challenged mice, we examined the level of the EMT markers including E-cadherin, N-cadherin, and α -SMA in lung tissue.

Immunofluorescent staining of lung sections found significantly decreased staining of E-cadherin and significantly increased expression of N-cadherin in the airway epithelium after the OVA challenge, as compared to that in the control mice (Fig. 3A). Airway smooth muscle mass as determined by immunostaining for α -SMA was significantly increased after the OVA challenge (Fig. 3A, B). Moreover, pretreatment with DEX and AZM significantly prevented alterations in EMT markers induced by the OVA challenge (Fig. 3A, B).

To further evaluate the effect of AZM on EMT, we performed qRT-PCR and western blotting in lung to determine the mRNA and protein expressions of E-cadherin, N-cadherin, and α -SMA. As we expected, the qRT-PCR results showed similar patterns. In detail, the expressions of N-cadherin and α -SMA significantly increased with the OVA challenge and were significantly suppressed by AZM, whereas E-cadherin was expressed in the opposite pattern (Fig. 3C–E) in mRNA levels

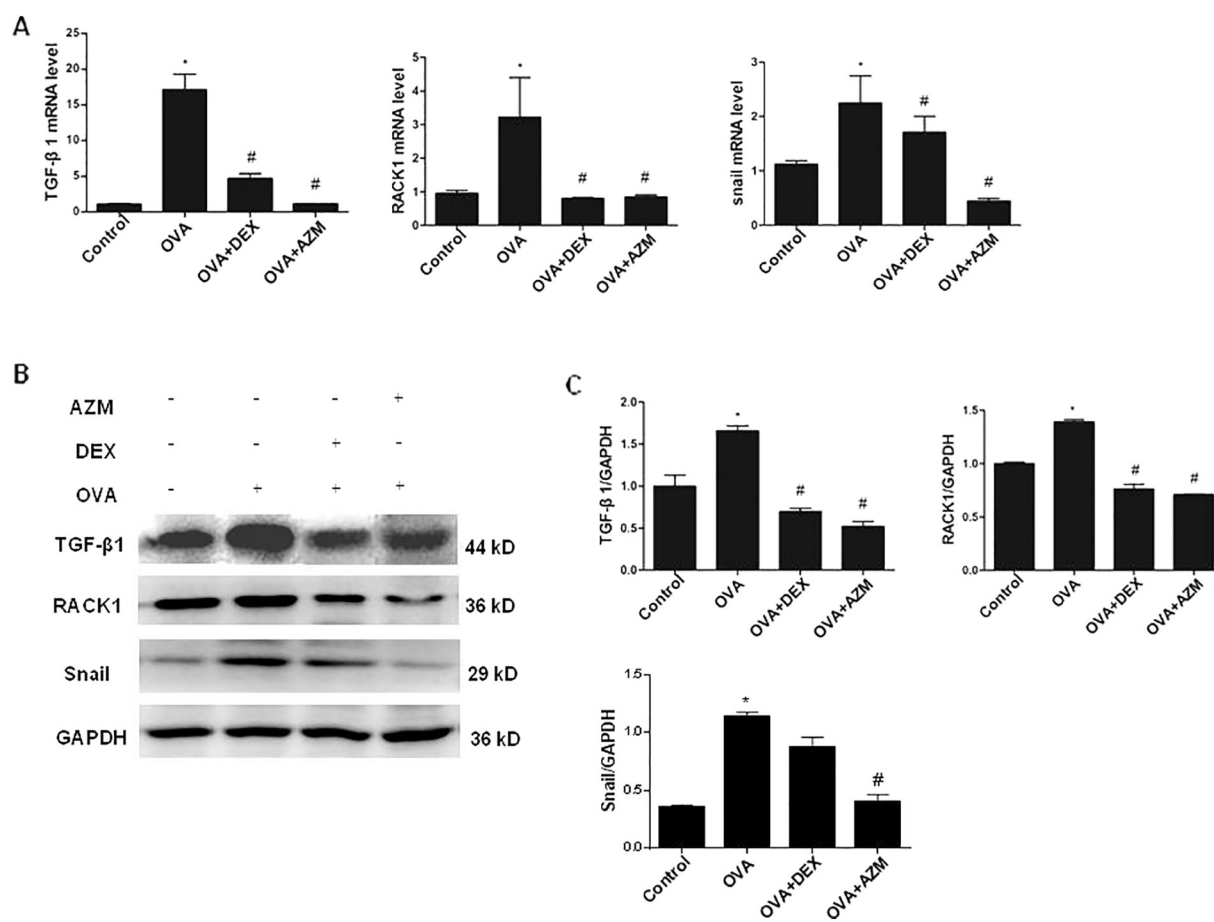


Fig. 4. Effects of azithromycin on TGF-β1/RACK1/Snail signaling pathway.

(A) The mRNA levels of TGF-β1, RACK1, and Snail were determined by quantitative real-time polymerase chain reaction (qRT-PCR). mRNA value = $2^{-\Delta\Delta Ct}$, $\Delta\Delta Ct = (Ct_{Target} - Ct_{GAPDH})$ of the treated mice - $(Ct_{Target} - Ct_{GAPDH})$ of the control mice. (B) The protein expressions of TGF-β1, RACK1, and Snail were analyzed by western blotting. GAPDH was used as an internal standard. (C) The relative levels of TGF-β1, RACK1, and Snail expressions in the lung. The results were expressed as the ratio of the investigated gene to GAPDH. The band intensity was analyzed by Bio-Rad Quantity One v4.62 software. Values are presented as mean $\pm$ S.E.M. * $P < 0.05$, versus control group ($n = 6$). # $P < 0.05$, versus OVA group ($n = 6$).

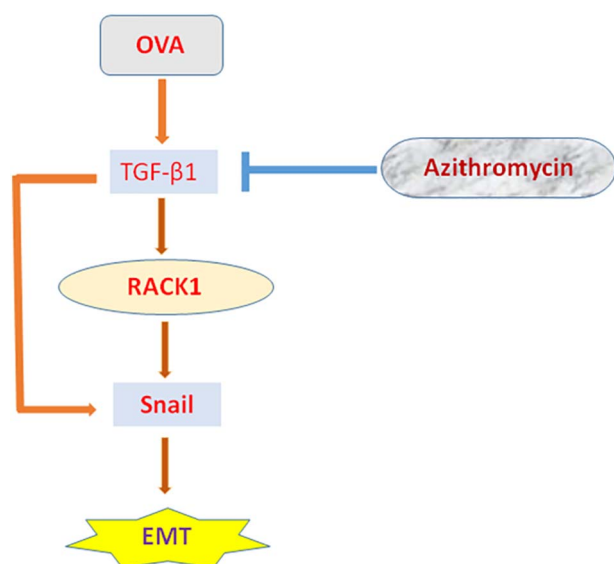


Fig. 5. Graphical summary of the results.

On exposure to OVA, snail was upregulated directly by TGF-β1 or indirectly by RACK1. Subsequently, Snail activated the EMT marker. Azithromycin suppresses airway EMT by inhibiting the TGF-β1/RACK1/Snail signaling pathway.

($P < 0.05$). Nevertheless, the E-cadherin and N-cadherin mRNA were not affected by DEX treatment ($P < 0.05$) (Fig. 3C–D). Furthermore, the protein levels of N-cadherin and α -SMA were significantly upregulated with OVA and reversed by AZM treatment ($P < 0.05$) (Fig. 3F, H–I). However, OVA, AZM, and DEX had no effect on E-cadherin expression (Fig. 3F, G), indicating that some N-cadherin and α -SMA up-regulating may be required for OVA-induced EMT, and AZM could regulate the tissue remodeling involved in EMT.

3.3. Azithromycin targets the TGF-β1/RACK1 signaling pathway to suppress EMT

TGF-β1 is a well-known key regulator of tissue remodeling in the airway epithelium through activation of EMT signals [17]. To elucidate the hypothesis that azithromycin-repressed EMT was coupled to a decreased TGF-β1 level, qRT-PCR and western blotting analysis for mRNA and protein expressions of TGF-β1 were performed, respectively. Consistent with our previous publication [2], azithromycin notably decreased TGF-β1 mRNA and protein levels from repetitive OVA challenged mice ($P < 0.05$) (Fig. 4A–C).

RACK1, a downstream target gene of TGF-β1 [14], has been demonstrated to promote upregulation of snail in primary HSCs [13]. To further examine whether the inhibitory effect of azithromycin on OVA challenge was associated with the reduction in RACK1, the mRNA and protein expression levels of RACK1 in lung were analyzed with qRT-PCR and western blotting. As shown in Fig. 4A–C, both the mRNA and protein expression levels of RACK1 were significantly elevated in OVA-

challenged mice compared with that in saline-challenged mice ($P < 0.05$), whereas AZM and DEX treatment notably reversed this elevation ($P < 0.05$).

Moreover, snail is a zinc-finger transcriptional repressor also directly activated by TGF- β 1 [11]. It was considered to downregulate E-cadherin expression and induce EMT by binding E-boxes [9]. We examined whether the OVA challenge promoted Snail expression and whether AZM terminated the EMT process via Snail. The mRNA and protein levels of Snail were significantly upregulated by the OVA challenge and suppressed by AZM ($P < 0.05$).

4. Discussion

Excessive airway epithelial damage followed by aberrant repair is believed to play an important role in asthma [18]. The EMT has been proposed as one of the mechanisms responsible for dysfunction of the epithelial barrier in asthma [6]. In agreement with previous reports [7], our data demonstrate that E-cadherin as an epithelial marker is significantly decreased, whereas α -SMA and N-cadherin as mesenchymal markers are significantly increased in OVA-challenged Balb/c mice.

Although glucocorticoids (GCs) possess potent anti-inflammatory, immunosuppressive properties and have been documented to block EMT initiated by TGF- β 1 in chronic rhinosinusitis with nasal polyps (CRSwNP) [19], adverse effects and the development of GC resistance limit its widespread and long-term use. Whereas, azithromycin has been reported to improve quality of life in symptomatic asthma, even as inhaled maintenance therapy [20], and is considered to be the useful add-on therapy in persistent asthma [21]. Our previous studies have demonstrated that azithromycin is an alleviator of airway inflammation and remodeling in OVA-challenged rats and cultured BEAS-2B cells [2,3]. Recent research suggests that EMT is a complex manifestation of epithelial plasticity and plays a critical role in airway remodeling in asthma [6]. In the present study, we investigated the possible role of azithromycin on airway EMT in vivo. Consistent with our previous findings in rats, our data showed that azithromycin effectively inhibited goblet cell hyperplasia and excessive deposition of collagen fibers, two characteristic components of airway remodeling in OVA-challenged Balb/c mice. Further, azithromycin administration significantly allayed the EMT process by recovering E-cadherin expression and suppressing α -SMA and N-cadherin induction in OVA-challenged Balb/c mice.

TGF- β 1 is a pleiotropic profibrotic cytokine that has been shown to be produced by epithelial cells to induce airway EMT in asthma [22]. Accumulating evidence indicates that in asthma [10], TGF- β 1 directly activates transcriptional factor Snail [23], which directly binds to E-boxes in E-cadherin promoters [9] and represses transcription of E-cadherin [8]. Although TGF- β /Smad signaling is regarded as the main pathway in EMT, TGF- β activates the non-Smad signaling pathway as well, including RACK1, which is the downstream target gene of TGF- β 1 [14]. In particular, a high level of RACK1 is necessary for regulatory T-cell function in patients with asthma [24]. Interestingly, a recent research has demonstrated that the activation of snail is mediated by RACK1 in primary HSCs [13]. Our data showed that mRNA and protein levels of TGF- β 1, RACK1, and Snail were notably elevated in OVA challenged mice, indicating the central role ascribed to TGF- β 1/RACK1/Snail in triggering EMT. Considering the role of azithromycin in recovering the reduction of E-cadherin level in gingival epithelial cells [15], we hypothesized that azithromycin exerted its efficacy via inactivation of key molecules in the TGF- β 1/RACK1/Snail signaling pathway. We found that snail, TGF- β 1, and RACK1 were significantly repressed by azithromycin in OVA-challenged Balb/c mice. Thus, we speculate that azithromycin may play a therapeutic role in airway EMT by blocking TGF- β 1/RACK1/Snail signaling.

In summary, our results indicate that azithromycin inhibits the TGF- β 1/RACK1/Snail signaling pathway and subsequently suppresses airway EMT and remodeling, thus leading to an improvement in OVA-induced asthma in Balb/c mice (Fig. 5).

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81470224 to Lihong Wan).

Abbreviations

OVA	ovalbumin
PAS	periodic acid-Schiff
HE	hematoxylin and eosin
Wat	airway wall thickness
Wam	bronchial smooth muscle thickness
AZM	azithromycin
DEX	dexamethasone
TGF- β 1	transforming growth factor- β 1
qRT-PCR	quantitative real-time polymerase chain reaction
EMT	epithelial-to-mesenchymal cell transition
RACK1	receptor for activated C-kinase1

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