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ORIGINAL RESEARCH

Curcumol Ameliorates Lung Inflammation and Airway Remodeling via Inhibiting the Abnormal Activation of the Wnt/β-Catenin Pathway in Chronic Asthmatic Mice

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Background: Curcumol exhibits anti-inflammatory effect, but its effect on chronic asthma lacked research. Therefore, this study explored the role of curcumol in asthma.

Methods: A chronic asthmatic mice model was established by ovalbumin induction. After treatment with curcumol, airway resistance in mice was detected by forced oscillation technique. The histopathological features of airway tissues, pulmonary inflammation, and inflammation cell recruitment in the bronchoalveolar lavage fluid (BALF) of mice were detected by hematoxylin-eosin staining. Collagen deposition in the airways of mice was examined by Masson staining. The secretion of ovalbumin-IgE, IL-4, IL-5, IL-13 in mouse serum and VEGFA secretion in BALF were analyzed by ELISA. Finally, the expressions of β-catenin, Wnt5a, VEGFA, TGF-β1, Fibronectin, and MMP-9 in mice lung tissues were determined by Western blot or immunohistochemical.

Results: Curcumol attenuated airway hyperresponsiveness, airway remodeling, and pulmonary inflammation in chronic asthmatic mice. Curcumol relieved collagen deposition in airway tissues, inflammation cell recruitment in BALF, and reduced the up-regulation of serum ovalbumin-IgE, IL-4, IL-5, and IL-13 and BALF VEGFA in chronic asthmatic mice. In addition, curcumol attenuated the up-regulated expressions of β-catenin, Wnt5a, VEGFA, TGF-β1, Fibronectin, and MMP-9 in the lung tissues of chronic asthmatic mice, but curcumol treatment did not show such effects on healthy mice.

Conclusion: Our findings revealed that curcumol could ameliorate lung inflammation and airway remodeling by inhibiting the abnormal activation of the Wnt/β-catenin pathway in chronic asthmatic mice, indicating that curcumol could be used as a novel anti-asthma drug for basic and clinical research.

Keywords: curcumol, chronic asthma, inflammation, airway remodeling, Wnt/β-catenin

Introduction

Asthma, alternatively known as bronchial asthma, is the most common chronic disease of the respiratory system.[1,2] In most cases, if not treated in time, acute asthma will evolve into chronic asthma, which could result in mortality.[1] In recent years, epidemiological studies at home and abroad have shown that the incidence of asthma is increasing annually.[2] According to the statistics, there are currently at least 300 million cases of asthma patients worldwide.[3] Currently, increasingly aggravated environmental pollution also contributes greatly to the high incidence

of asthma.[2,4] At present, glucocorticoids are the first-line choice for asthma treatment, but this treatment can cause side effects in patients, especially for those with chronic asthma.[5,6] Therefore, it is of great significance to better understand the pathogenesis of chronic asthma and explore an effective targeted therapy for treating chronic asthma.

Although many inducing factors of asthma have been discovered, the pathogenesis of asthma still remained poorly understood.[2] At present, modern medical research has found that chronic asthma may be resulted from chronic airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling (AR).[7,8] Increases of interleukin (IL)-4, IL-5, IL-13 are related to inflammation in asthma mice.[9] The development of eosinophilic airway inflammation, AHR and airway remodeling could be exacerbated by upregulating the expression levels and promoting the activity of matrix metalloproteinase-9 (MMP-9) in rats with chronic asthma.[10]

Previous research based on in vitro experiments has reported that curcumol can suppress lipopolysaccharide-induced inflammation in macrophages.[11] Curcumol is a Traditional Chinese Medicine (TCM) monomer extracted from Curcuma zedoariae, Curcuma curcumae, or Guangxi Zedoary turmeric.[12] Multiple biological activities of curcumol have been identified by a large number of in vivo and in vitro studies. For example, curcumol could induce growth inhibition and apoptosis of non-small cell lung cancer cells;[13] Peng et al found that the effect of curcumol on epigenetic regulation is realized by modulating DNA methyltransferase and histone deacetylase;[14] the regulatory effects of curcumol on pulmonary fibrosis, oxidative stress, and cancer cell metastasis are also reported.[15–17] Although the anti-inflammation effect of curcumol has been reported, the role of curcumol on chronic asthma is still unclear.

Wnt/β-catenin pathway is a signal transduction pathway triggered by the binding of ligand protein Wnt and membrane protein receptors, and it is involved in a majority of biological processes.[18–21] Binding of Wnt proteins to cell surface receptors leads to stabilization of the cytosol and translocation of β-catenin to the nucleus.[22] After that, β-catenin stimulates the transcription of target genes in cooperation with T-cell-factor/lymphoid enhancer-binding factor.[23] Studies based on animal researches also proved that the Wnt/β-catenin pathway regulates the process of AR in asthma.[24,25] The Wnt/β-catenin signaling pathway is high-expressed and regulates the development of airway remodeling in chronic asthma.[25] In addition, evidence showed that curcumol could regulate the activation of the Wnt/β-catenin pathway to further affect the progression of lung cancer.[26] Therefore, we hypothesized that curcumol exerted an inhibitory effect in chronic asthma, which is associated with a reduced signaling in the Wnt/β-catenin pathway.

In the present study, we employed ovalbumin (OVA)-induced chronic asthma model to investigate the role of curcumol on chronic asthma and the related mechanism.

Methods

Ethics Statement

Animal experiments were authorized by the Committee of Experimental Animals of Hengdian Wenrong's Hospital (Z20190921H). All experiments were performed in Hengdian Wenrong's Hospital and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Wenzhou Medical University.

Mice

Fifty female BALB/c mice (weight: 20–25 g, age: 6–8 weeks) were obtained from SLAC Laboratory Animal Technology (Shanghai, China). Every 5 mice were placed in the same cage, and all the mice were raised in the same SPF room under a 12-h light/dark cycle with free access to food and drink. Before experiment, the mice were acclimatized for 5 days.

Curcumol Treatment

Curcumol with a molecular weight of 236.35 and a purity of >99.87% was purchased from Targetmol (T2853, Boston, USA, https://www.targetmol.com/) and preliminarily stored at −80°C. Before use, it was heated at 37°C for 1 min and then dissolved in double distilled water to the administration dose used in animal experiments.

Establishment of Chronic Asthmatic Model

An asthma model was established according to a previous research.[25] The model construction consisted of two stages (Figure 1A), which were sensitization and maintenance stages. For the sensitization stage (the first 2 weeks), on the 1st and 8th day, the mice were intraperitoneally injected with 200 μL of 0.9% NaCl solution containing 75 μg OVA (vac-stova, Invivogen, Toulouse, France) and 2 mg of $Al(OH)_3$ (R003291, Rhawn, Shanghai, China, https://www.rhawn.cn/). For the maintenance stage (week 3 to week 12), the mice were intranasally injected with 20 μL

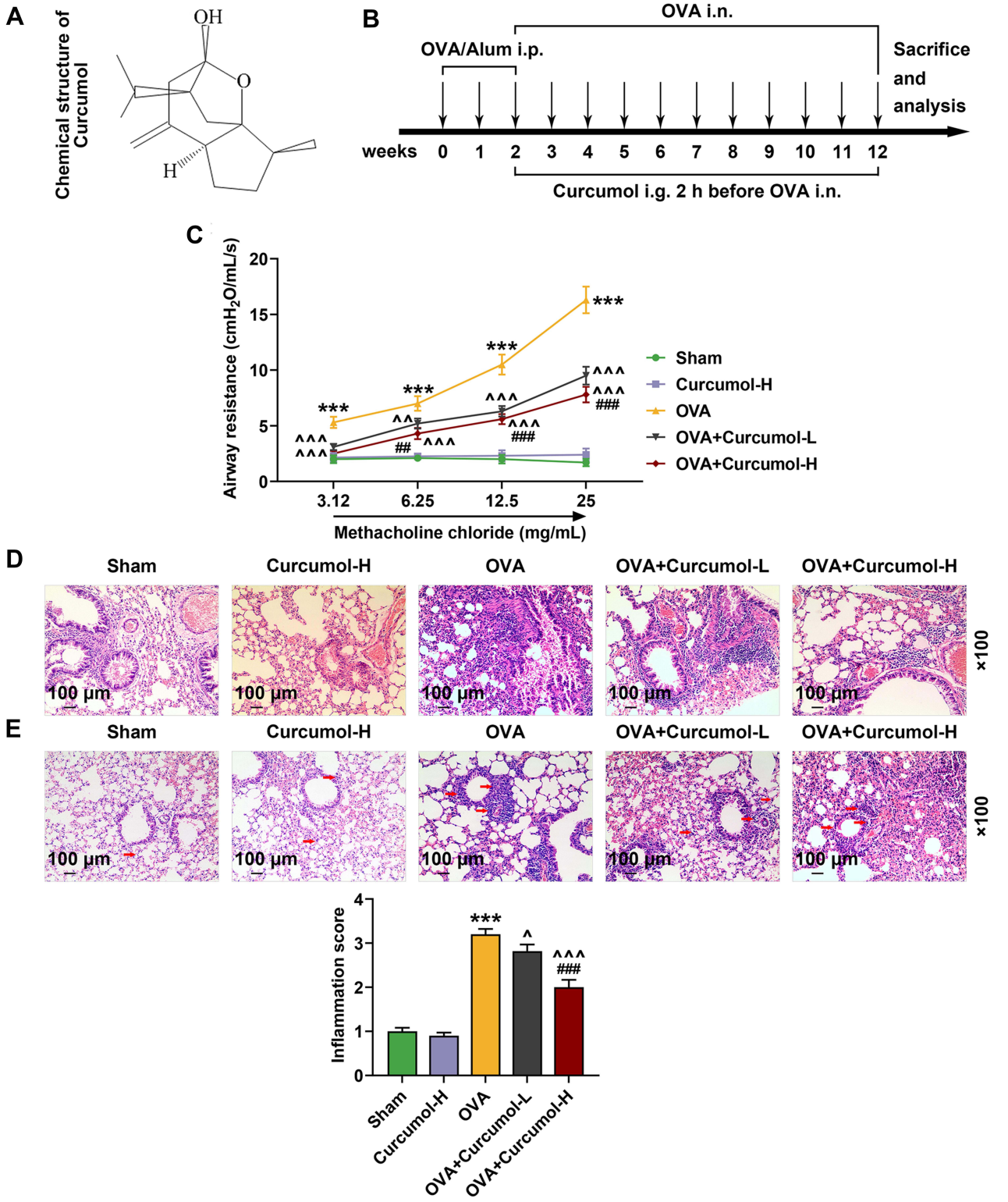

Figure 1 Curcumol attenuated AHR, AR, and pulmonary inflammation in chronic asthmatic mice. (**A**) The chemical structure of curcumol. (**B**) Experimental layout to develop a mice model of chronic asthma. (**C**) Airway resistance of the mice was detected by the forced oscillation technique. (**D**) Airway tissues of the mice were stained by hematoxylin-eosin to detect AR in the mice. (**E**) Lung tissues of the mice were stained by hematoxylin-eosin to detect pulmonary inflammation in the mice, the red arrows indicate an inflammatory infiltrate.

Notes: $^{***}P < 0.001$, vs Sham; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, vs Curcumol-H; $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$, vs OVA.

Abbreviations: AHR, airway hyperresponsiveness; AR, airway remodeling; OVA, Ovalbumin; Curcumol –H, high dose of curcumol-H; Curcumol-L, low dose of curcumol.

of OVA (50 μg/kg) in 0.9% NaCl solution twice a week for 10 weeks from the 15th day of the experiment.

Treatment of the Experimental Mice

The experimental mice were randomly divided into the following 5 groups (n=10): the Sham group, the Curcumol-H group, the OVA group, the OVA+Curcumol-L group, and the OVA+Curcumol-H group.

The flow chart of the treatment is shown in Figure 1A. In brief, for the Sham group, the mice were intraperitoneally injected with 200 μL of 0.9% NaCl solution without OVA and $Al(OH)_3$ in the sensitization stage. In the maintenance stage, all OVA administrations of mice were replaced by 20 μL of 0.9% NaCl solution. For the Curcumol-H group, on the basis of the Sham group, the mice were orally administrated with 20 μL of Curcumol (80 mg/kg, in 0.9% NaCl solution) 2 h before each NaCl administration in the maintenance stage. For the OVA group, the administration of the mice was the same as that adopted in the establishment of the chronic asthmatic model. For the OVA+Curcumol-L and OVA+Curcumol-H groups, on the basis of the OVA group, the mice were orally administrated with 20 μL of curcumol (20 or 80 mg/kg, in 0.9% NaCl solution) 2 h before each OVA administration in the maintenance stage.

On the last day of administration, airway hyperresponsiveness (AHR) of mice was first examined. After the mice were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (B005, JianCheng Bio, Nanjing, http://www.njjcbio.com/) at a dose of 50 mg/kg, the cells in bronchoalveolar lavage fluid (BALF), blood, lung tissues, and airway tissues were collected.

Assessment of AHR

The detection of AHR was conducted 24 h after the last OVA or curcumol administration in the mice by the forced oscillation technique as previously described.[27] In brief, after the mice were anesthetized, a catheter was inserted into the trachea of the mice. The catheter was then connected with a MS-IOS pulmonary function detector (JAEGER, Lüdenscheid, Germany, https://www.jaeger-poway.com/) and mechanically ventilated. Airway responsiveness was determined by increasing the doses of inhaled methacholine chloride. Data were collected every 1 min and the lung function values of airway resistance (cm H_2O/mL/s) were plotted to reflect the effects of methacholine chloride doses.

Bronchoalveolar Lavage Fluid (BALF) Collection

After the catheter was inserted into the trachea of the mice, 200 μL of cold PBS (10010049, Gibco, MA, USA) was injected into the lung using a syringe. Then, BALF samples were collected into the syringe. After the BALF samples were centrifuged for 10 min (300 × *g*), the supernatants were stored at −80°C for ELISA experiment. The precipitate, which was the cells in BALF, was further re-suspended in 200 μL of PBS and used for hematoxylin-eosin staining. Cells were counted by automatic cell counting and vitality analysis system.

Hematoxylin-Eosin Staining

Lung and airway tissues were fixed with 4% paraformaldehyde (R008069, Rhawn) for 24 h at room temperature. Then the samples were washed overnight with tap water and dehydrated with gradient ethanol (E809057, Macklin, Shanghai, China) and double distilled water. Subsequently, the tissues were embedded into paraffin, sectioned, and dewaxed. Then the tissues or cells in BALF were stained by hematoxylin (B25380, Yuanye, Shanghai, China) for 10 min and further incubated with hydrochloric acid alcohol (AR0038, Boster, Wuhan, China, http://www.boster.com.cn/index/index.html) for 5 min. Then the samples rested in 50°C warm bath until the tissues appeared blue, and were further stained with eosin (C0109, Beyotime) for 1 min at room temperature. After soaking the samples in gradient ethanol again for dehydration, the sample slices were made transparent by soaking in xylene (R017750, Rhawn). Finally, the sample slices were sealed with neutral gum (N116470, Aladdin, Shanghai, China) and the image of each section was collected under a DMLA full automatic microscope (Leica, Solms, Germany) at a magnification of 100 ×.

Masson Staining

The airway tissues were fixed with 4% paraformaldehyde for 24 h at room temperature. Then the tissues were washed overnight with tap water and dehydrated by gradient ethanol, which was prepared with different volume of 100% ethanol and double distilled water. Subsequently, the tissues were paraffin-embedded, sectioned, and dewaxed. After being stained with hematoxylin for 10 min, the tissues were further incubated with hydrochloric acid alcohol for 5 min. Then the tissues were further dyed with Masson solution (D026-1-2, Jiangcheng Bio, http://

www.njjcbio.com/) for 5 min, 1% phosphomolybdic acid aqueous solution (G3472, Solarbio) for 3 min, and toluidine blue (T818873, Macklin) for 5 min at room temperature. After being soaked in gradient ethanol again for dehydration, the tissue slices were made transparent by rinsing in xylene. Finally, the tissue slices were sealed with neutral gum and the image of each section was collected under a DMLA full automatic microscope (Leica, Solms, Germany) at a magnification of 100 ×.

ELISA

The serum level of OVA-IgE, IL-4, IL-5, and IL-13 of the mice and the level of VEGFA in BALF were detected by ELISA. The mouse OVA-IgE (ml063584), mouse IL-4 (ml064310), mouse IL-5 (ml063157), mouse IL-13 (ml063123), and mouse VEGFA kits (ml076303) were obtained from Enzyme-linked Biotechnology (Shanghai, China). In brief, 50 μL of the test samples (serum or BALF) and standard samples of known concentrations were added into the designated wells of the 96-well plate, which was specially used for detecting these factors. Then, all the wells were added with 50 μL of biotin-labeled antibody and incubated for 1 h at 37°C in the dark. After adding 200 μL of wash buffer, the plate was washed and slightly shaken for 30 s for three times. Then, 80 μL of affinity chain enzyme HRP was added into each well and incubated at 37°C for 30 min. Next, 50 μL of A solution and B solution were further added into each well for another 20-min incubation. Finally, 50 μL of stop buffer was added into each well and the absorbance was measured at 450 nm using an Imark ELISA reader (Bio-Rad, CA, USA).

Immunohistochemistry

The airway tissues were fixed with 4% paraformaldehyde for 24 h at room temperature. Then the tissues were washed overnight under tap water and dehydrated with gradient ethanol, which was prepared with different volumes of 100% ethanol and double distilled water. Subsequently, the tissues were paraffin-embedded, sectioned, and dewaxed. After soaking the tissues in 3% hydrogen peroxide (C804187, Macklin) for 25 min and in citrate antigen repair solution (AR0024, Boster, Wuhan, China), the tissue slices were heated in a microwave oven (BE01, Galanz, Guangdong, Shanghai) for 8 min. Then the tissue slices were naturally cooled at room temperature and then incubated with β-catenin antibody (1:250, ab32572, Abcam) overnight at 4°C. The second day, the slices were further incubated with goat-anti-rabbit IgG (1:30000, ab205718, Abcam) antibody for 1 min, followed by incubation with the DBA reagent (SFQ004, 4A Biotech, Beijing, China) for 30 min. After being washed for 1 min with tap water, the tissues were stained with hematoxylin for 4 min. Then the tissues were soaked in gradient ethanol again for dehydration and made transparent by rinsing in xylene. Finally, the tissue slices were sealed with neutral gum and the image of each section was collected under a DMLA fully automatic microscope (Leica, Solms, Germany) at a magnification of 100 ×.

Western Blot Assays

NP-40 (P0013F) was purchased from Beyotime for the extraction of total protein in airway tissues. 25 mg of airway tissues stored at −80°C were lysed with 300 μL of NP-40 solution at 4°C. After the tissues were centrifuged for 30 min at 4°C and 14,000 × *g*, the total protein was obtained. Then, a BCA kit (P0009, Beyotime) was further applied for the determination of protein concentration. After the protein concentration in each group was determined, 25 μg of protein in each group and 4 μL of marker (PR1910, Solarbio) were transferred into the designed lane of the 10% SDS-PAGE gels (P0052A, Beyotime). Then, the protein swam vertically down inside the gels under 100 volt for 120 min, and then transferred to the surface of 0.45 μm membranes (ISEQ00010, Millipore, MA, USA). The pore without target proteins on the membranes was blocked with 5% non-fat milk, and then the membranes were incubated with relative primary antibodies at 4°C overnight for target protein detection. The relative primary antibodies were Wnt5a (1:1500, 42kD, ab229200, Abcam), β-catenin (1:1000, 86kD, ab32572, Abcam), VEGFA (1:2500, 27kD, ab52917, Abcam), TGF-β1 (1:1000, 44kD, ab92486, Abcam), Fibronectin (1:1500, 262kD, ab2413, Abcam), MMP-9 (1:2500, 92kD, ab38898, Abcam), and GAPDH (1:5000, 36kD, ab181602, Abcam). The second day, the rabbit secondary antibody (1:20,000, ab205718, Abcam) was applied to further incubate with all the membranes for 2 h at room temperature. Finally, each membrane was covered with 200 μL of detection solution (P0019, Beyotime), and the image signal was detected using Bio-Rad ChemiDoc™ XRS+ System and the Image Lab 3.0 Software (Bio-Rad, CA, USA).

Statistical Analysis

All the statistical analyses were conducted using GraphPad 8.0 software. Comparison between multiple groups was analyzed

using One-way ANOVA. Mean ± SD was used to describe the statistical data. $P < 0.05$ was considered as statistically significant. Shapiro–Wilk test was used to assess data distribution. Q-Q plot was performed by IBM SPSS Statistics 20.

Results

Curcumol Attenuated AHR, AR, and Pulmonary Inflammation in Chronic Asthmatic Mice

The chemical structure of curcumol is shown in Figure 1A. To explore the effect of curcumol on chronic asthma, we first established an OVA-induced chronic asthma mice model (Figure 1B). As AHR is the direct symptom of asthma, we then examined airway resistance in the mice. As shown in Figure 1C, the increase of methacholine chloride did not affect the airway resistance in the Sham and curcumol-H groups, indicating that curcumol-H had no enhancing effect on airway resistance in those mice. However, airway resistance in the OVA group was remarkably enhanced as compared with the Sham groups ($P < 0.001$), while in the OVA+ curcumol-L and OVA+curcumol-H groups, curcumol treatment attenuated OVA-induced airway resistance as compared with the OVA group ($P < 0.01$), although airway resistance in the OVA +curcumol-H group was stronger than that in the curcumol-H group ($P < 0.001$). This result revealed that curcumol could relieve AHR in chronic asthma mice. Airway resistance is closely associated with AR, and therefore we subsequently detected the AR condition in airway tissues of the mice in all groups by hematoxylin-eosin staining. The results revealed that the airway tissues were normal in the Sham and curcumol-H groups, and the airway wall and smooth muscle layer of mice in the OVA group were significantly thickened, suggesting the occurrence of AR in the mice with OVA-induced chronic asthma, but in the OVA+ curcumol-L and OVA+curcumol-H groups, curcumol attenuated the OVA-induced AR (Figure 1D). Mechanically, we also detected pulmonary inflammation in the lung tissues by hematoxylin-eosin staining (Figure 1E), and the results showed that the lung tissues were normal and had no inflammation in the Sham and curcumol-H groups, and inflammatory infiltration in lung tissues in the OVA group was significantly enhanced as compared with the Sham group ($P < 0.001$). In the OVA +curcumol-L and OVA+curcumol-H groups, curcumol treatment attenuated the OVA-induced pulmonary inflammation as compared with the OVA group ($P < 0.05$).

Curcumol Attenuated Collagen Deposition, Inflammatory Cell Recruitment, and the Up-Regulation of OVA-IgE, IL-4, IL-5, IL-13, VEGFA, and β-Catenin in Chronic Asthmatic Mice

To further verify the present findings, a series of experiments were conducted. The collagen deposition in lung tissues of the mice was examined by Masson staining (Figure 2A), and the results showed that collagen deposition barely appeared in the lung tissues of mice in the Sham and curcumol-H groups, but it was notably increased in the OVA group. Collagen deposition induced by OVA was then found to be attenuated by curcumol treatment in the OVA+curcumol-L and OVA+curcumol-H groups. Then, hematoxylin-eosin staining was conducted to analyze inflammatory cell recruitment in BALF. As shown in Figure 2B–C, there was a small number of inflammatory cells in the mice BALF of the Sham and curcumol-H groups, but the proportion of inflammatory cells was greatly increased in the OVA group as compared with the Sham group ($P < 0.001$). We also found that the increase of inflammatory cells induced by OVA was attenuated by curcumol treatment in the OVA+curcumol-L and OVA+curcumol-H groups. Furthermore, OVA-IgE secretion in serum, VEFGA secretion in BALF, and serum IL-4, IL-5, IL-13 secretion in the mice were detected (Figure 2D–H), and the results exhibited that OVA-IgE, VEGFA, IL-4, IL-5, and IL-13 levels in the curcumol-H group had no change as compared with those in the Sham group, but the levels of these factors were sharply increased in the OVA group when compared with those in the Sham group ($P < 0.001$). In the OVA+curcumol-L and OVA+curcumol-H groups, the high levels of the factors induced by OVA were reduced by curcumol treatment as compared with those in the OVA group ($P < 0.05$). The levels of these factors in the OVA+curcumol-H group were higher than those in curcumol-H group ($P < 0.05$). Finally, we used immunohistochemistry to detect the expression of β-catenin in the lung tissues of the mice (Figure 2I), and found that β-catenin expression had no difference between the Sham group and the curcumol-H group, but it was obviously up-regulated in the OVA group. The promoted expression of β-catenin induced by OVA was attenuated by curcumol treatment in the OVA+curcumol-L and OVA+curcumol-H groups.

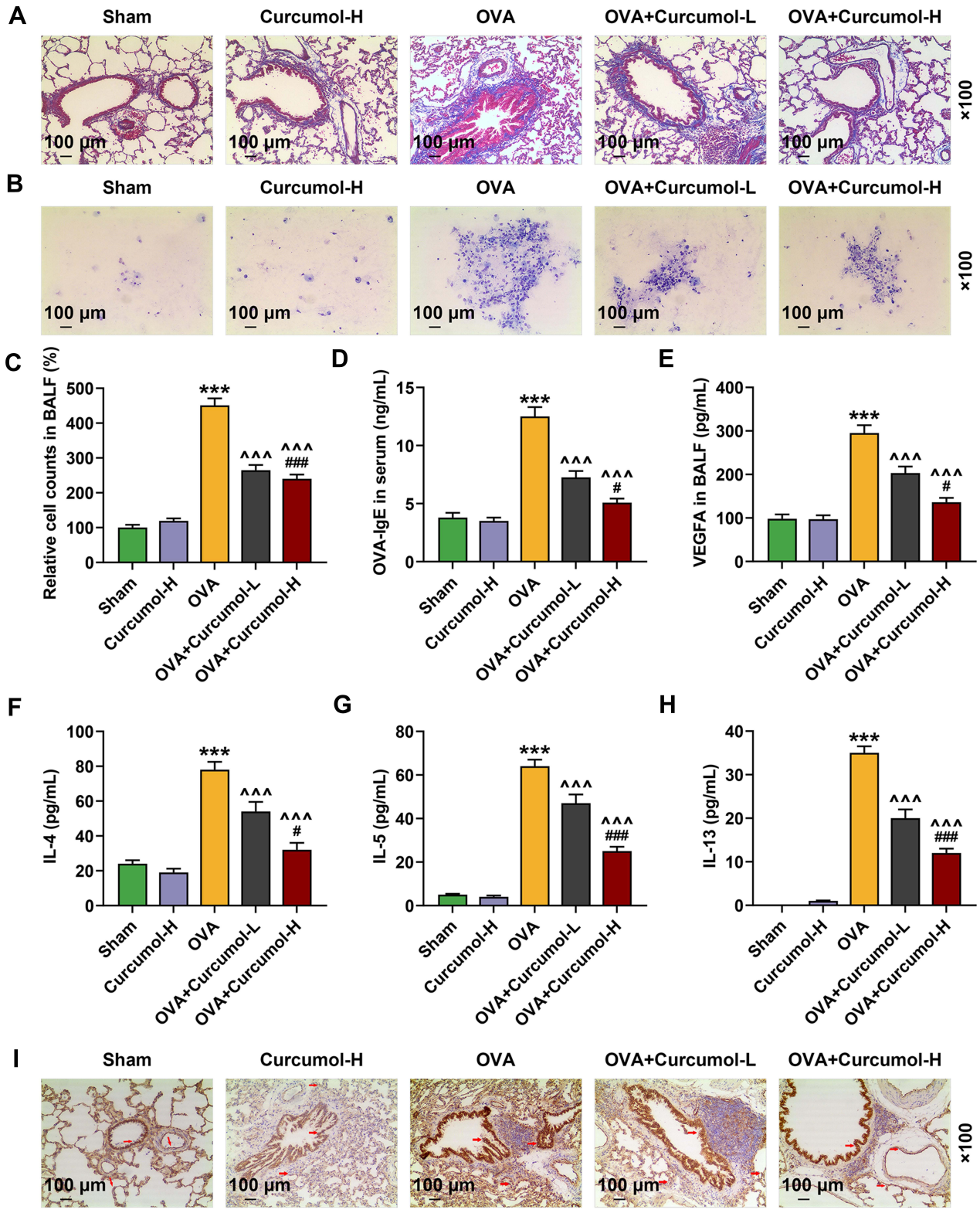

Figure 2 Curcumol attenuated collagen deposition, inflammatory cell recruitment, and the up-regulation of OVA-IgE, IL-4, IL-5, IL-13, VEGFA, and β-catenin in chronic asthmatic mice. (**A**) The mice airway tissues were stained by Masson to detect collagen deposition in the tissues. (**B-C**) The cells in BALF were stained by hematoxylin-eosin staining to examine the recruitment of inflammatory cells in mice BALF. (**D**) The secretion level of OVA-IgE in mice serum was detected by ELISA. (**E**) The secretion of VEGFA in mice BALF was detected by ELISA. (**F-H**) The secretion levels of IL-4 (**F**), IL-5 (**G**), and IL-13 (**H**) in mice serum were detected by ELISA. (**I**) The expression of β-catenin in mice lung tissues were detected by immunohistochemical. Arrows pointed to the representative staining of β-catenin.

Notes: $^{***}P < 0.001$, vs Sham; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$, vs Curcumol-H; $^{\wedge\wedge\wedge}P < 0.001$, vs OVA.

Abbreviations: OVA, ovalbumin; BALF, bronchoalveolar lavage fluid; Curcumol –H, high dose of curcumol-H; Curcumol-L, low dose of curcumol.

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Curcumol Attenuated the Abnormal Activation of the Wnt/β-Catenin Pathway and the Abnormal Expressions of VEGFA, TGF-β1, Fibronectin, and MMP-9 in Chronic Asthmatic Mice

We further detected the activation of the Wnt/β-catenin pathway in the mouse lung tissues. As shown in Figure 3A–C, the expressions of Wnt5a and β-catenin in the curcumol-H group showed no difference as compared with the Sham group, while the expressions of Wnt5a and β-catenin in the OVA group were remarkably up-regulated as compared with the Sham group ($P < 0.001$). Furthermore, in the OVA+curcumol-L and OVA+curcumol-H groups, curcumol treatment reversed the up-regulated expressions of Wnt5a and β-catenin as compared with the OVA group ($P < 0.05$). In addition, the expressions of Wnt5a and β-catenin in the OVA+curcumol-H group were higher than those in the curcumol-H group ($P < 0.05$). These results revealed that the Wnt/β-catenin pathway was greatly activated in chronic asthmatic mice, and that curcumol treatment could attenuate the abnormal activation of the Wnt/β-catenin pathway. Finally, we detected the expression changes of the down-stream factors in the Wnt/β-catenin pathway (Figure 3D–H). Similarly, the expressions of VEGFA, TGF-β1, Fibronectin, and MMP-9 in the curcumol-H group showed no difference as compared with the Sham group, but the expressions of these factors in the OVA group were promoted when compared with the Sham group ($P < 0.001$). Furthermore, in the OVA+curcumol-L and OVA+curcumol-H groups, curcumol treatment downregulated the expressions of these factors as compared with the OVA group ($P < 0.05$). In addition, the expressions of these factors in the OVA+curcumol-H group were higher than those in the curcumol-H group ($P < 0.05$).

Discussion

The present study investigated the effects and mechanisms of curcumol on asthma. After the OVA-induced chronic asthma mice model was established, curcumol was applied to treat these mice. Then, we found that curcumol attenuated the airway hyperresponsiveness, airway remodeling, pulmonary inflammation of chronic asthmatic mice. The collagen deposition in airway tissues and the inflammation cell recruitment in BALF were also reduced by curcumol treatment. In addition, curcumol treatment further attenuated the abnormally activated Wnt/β-catenin pathway and related factors in this pathway. Our findings revealed that curcumol could ameliorate lung inflammation and airway remodeling by inhibiting the abnormal activation of the Wnt/β-catenin pathway in chronic asthmatic mice.

As a natural triterpenoid monomer, curcumol was previously proven to possess multiple biological activities, including anti-oxidation, anti-cancer, anti-microbial, and neuroprotection.[12] In addition, anti-inflammation effect of curcumol on suppressing lipopolysaccharide-induced inflammation of macrophages has also been also confirmed.[11] Currently, little attention has been paid to the potential role of curcumol on the inflammation in chronic asthmatic. Therefore, we established a chronic asthmatic mouse model by stimulating healthy mice with OVA to further examine the mechanisms.

As one of the three main characteristics (AHR, airway inflammation, and AR) of chronic asthma,[1,4,28] AHR is caused by airway inflammation and AR.[29] Hence, we examined the changes of airway resistance immediately after the asthma mice model was treated with curcumol, and found that the elevated airway resistance in chronic asthmatic mice was relieved by curcumol. In addition, curcumol had no effect on airway resistance in healthy mice, indicating that curcumol could alleviate AHR in chronic asthmatic mice. As a direct cause of pulmonary dysfunction and severe AHR, AR refers to changes in airway structure such as airway wall thickening, collagen deposition, subepithelial fibrosis, and narrowing of the airway lumen.[8] In this study, we also found that thickening of airway wall and collagen deposition in chronic asthmatic mice were relieved by curcumol; moreover, curcumol had no effect on AR in healthy mice, indicating that curcumol could inhibit AR in chronic asthmatic mice. Furthermore, as the pathological basis of asthma, chronic airway inflammation is the main cause of AR.[8,30] Our results revealed that curcumol mitigated pulmonary inflammation and the inflammatory cell recruitment in BALF. In addition, the inflammation reaction of asthma is mainly related to IgE-mediated type I allergic reaction; therefore, the secretion of IgE and related inflammation-related factors (IL-4, IL-5, IL-13) is up-regulated in the serum of asthma patients.[31] We then further detected the OVA-IgE and the secretion of these factors. The results confirmed that curcumol mitigated the up-regulated serum OVA-IgE, IL-4, IL-5, and IL-13 in chronic asthmatic mice. The above evidence indicated that curcumol,

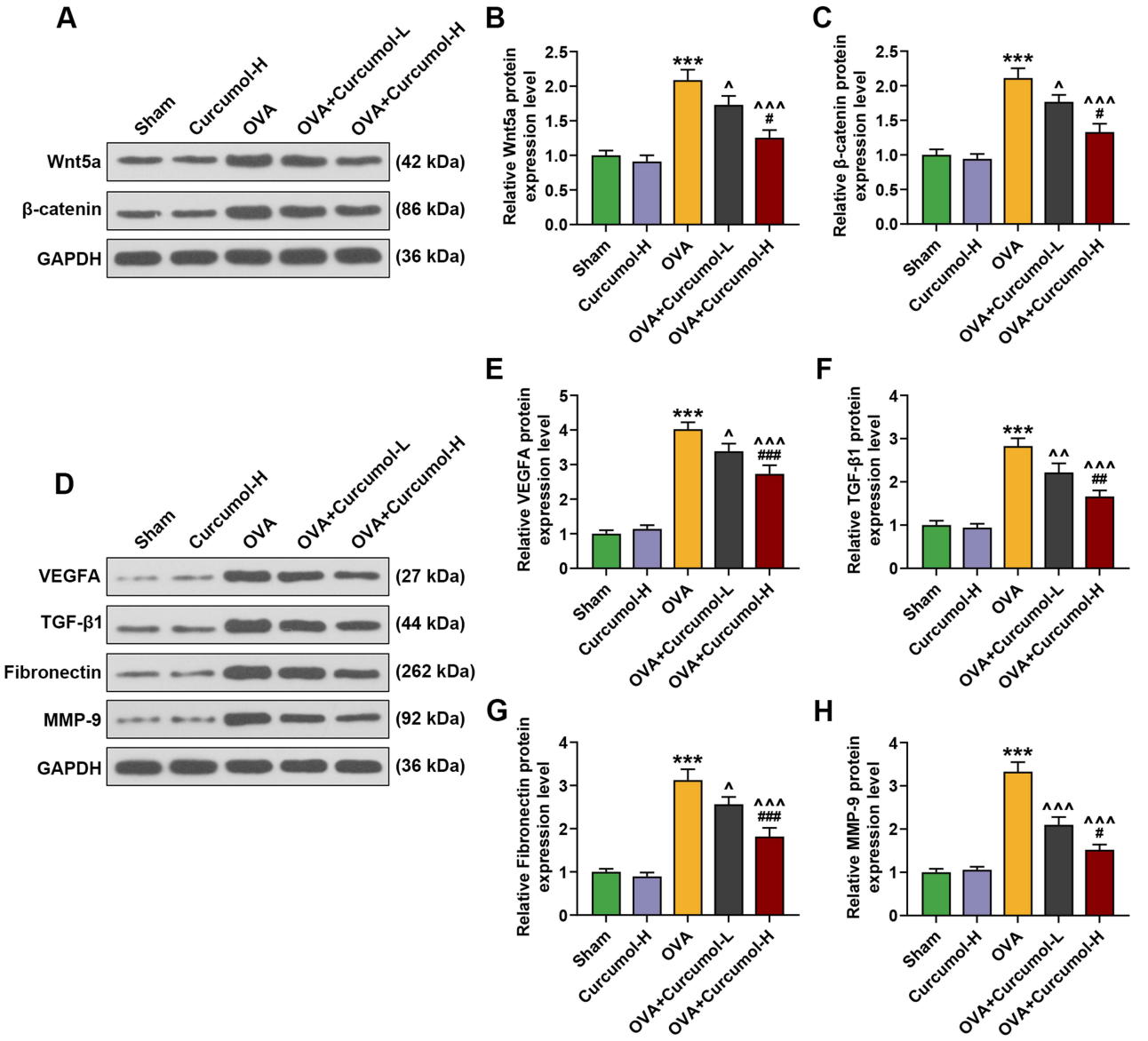

Figure 3 Curcumol attenuated the abnormal activation of the Wnt/β-catenin pathway and the abnormal expressions of VEGFA, TGF-β1, Fibronectin, and MMP-9 in chronic asthmatic mice. (**A–C**) The expressions of Wnt5a and β-catenin in mice lung tissues were detected by Western blot; GAPDH was an internal control. (**D–H**) The expressions of VEGFA, TGF-β1, Fibronectin, and MMP-9 in mouse lung tissues were detected by Western blot; GAPDH was used as an internal control.
Notes: $^{***}P < 0.001$, vs Sham; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, vs Curcumol-H; $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.01$, vs OVA.
Abbreviations: OVA, ovalbumin; Curcumol –H, high dose of curcumol-H; Curcumol-L, low dose of curcumol.

which had no side effect on healthy mice, attenuated AHR, AR, and airway inflammation in chronic asthma mice.

Many factors and signaling pathways are involved in the pathological progression of asthma. During airway inflammation and AR, hypertrophy and proliferation of smooth muscle cells, transformation of fibroblasts into myofibroblasts, and accumulation of extracellular matrix are regulated by related factors such as VEGFA, TGF-β1, Fibronectin, and MMP-9.[30,32–37] To further verify the findings in the current study, we detected the expressions of these factors. Interestingly, the results showed that curcumol attenuated the up-regulated expressions of VEGFA, TGF-β1, Fibronectin, and MMP-9 in the lung tissues of chronic asthmatic mice. Researches have increasingly proven that the Wnt/β-catenin pathway modulated the progression of asthma.[23,24,27] In addition, evidence indicated that curcumol could regulate the activation of the Wnt/β-catenin pathway to further affect the progression of lung cancer.[26] We detected the

expressions of the key factors in this pathway, and found that curcumol further attenuated the abnormal activation of the Wnt/β-catenin pathway in chronic asthmatic mice. However, whether canonical or non-canonical Wnt/β-catenin pathway functioned in chronic asthma, and the safe dose of curcomol remained to be further determined, which were also the limitations of this research.

To conclude, the findings in this research revealed that curcumol could ameliorate lung inflammation and airway remodeling, and curcomol was associated with lower Wnt5a expression, which could explain the symptom relief of the chronic asthma model.

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Disclosure

The authors declare no conflicts of interest.

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