Original Article
JNJ7777120, the histamine receptor 4 antagonist, decreases the allergic remodeling and Th2 inflammation in a rat model of allergic rhinitis

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Abstract: JNJ7777120 is a high-affinity specific inhibitor of histamine receptor 4 (HR4) was found have therapy function of allergic rhinitis. Our study investigated its function in ameliorating allergic remodeling and Th2 inflammation in a rat model. Rats sensitized to ovalbumin were chronically challenged by dripping into the nostril for and continued for 8 weeks. During this period, animals were treated with JNJ7777120 nasal drop. Biomarkers of IL-6 and IL-2 level were assessed to demonstrate the polarization of T helper 1 (Th1)/T helper 2 (Th2) inflammation both in nasal mucosa tissue and blood. Hematoxylin-eosin staining (HE) and Masson's trichrome (MT) and periodic acid Schiff (PAS) analysis for nasal turbinate mucosa were studied. Results showed that, compared with OVA allergic group, administration of JNJ7777120 significantly decreased the Th2-dominated reaction and increased Th1-dominated reaction and reduced the accumulating of eosinophils, decreased the subepithelial collagenization, and repaired the epithelium. These data indicate that JNJ7777120 may play a protective role in reducing nasal mucosa Th2- associated inflammation and allergic remodeling in rat allergic rhinitis.

Keywords: JNJ7777120, allergic rhinitis, remodeling, inflammation, animal model, histamine receptor 4

Introduction

Allergic rhinitis (AR) is defined as the nasal membrane inflammation triggered by specific IgE, accompanied with the increasing world prevalence and heavy economic burden [1]. Hygiene hypothesis tells that the polarization of T helper 1 (Th1)/T helper 2 (Th2) reactions caused AR. Eosinophils and mucus cell metaplasia and other structure changes in nasal mucosa are termed as tissue remodeling also associated with allergic rhinitis [2]. To date, many studies of AR were targeted on the Th2-dominated inflammation or remodeling respectively. However, the ideal therapy regimen of AR should focus on both of inflammation reaction and tissue remodeling.

Histamine is one of endogenous chemical substances that are released at the beginning of the allergic reaction and initialed the allergic cascade reaction [3]. The histamine receptor 4 (HR4), the fourth found receptor of histamine shown to be involved in almost all allergy related immune cells, including eosinophils, T cells, dendritic cells, basophils, mast cells and etc [4, 5]. JNJ7777120, a specific antagonist of HR4, could inhibit nasal symptoms by intranasal administration and diminished Th2 reaction with the evidence of decreased expression of IL-4 and increasing expression of IFN-gamma in nasal lavage fluid [6, 7]. Our study tried to study the effect of JNJ7777120 both on AR-associated inflammation and remodeling by AR animal model, and reveal whether there is a link between Th2 associate inflammation and structure by HR4 pathway.

Materials and methods

Chemical drug and reagents

Reagents used in the current study, including ovalbumin (OVA; chicken egg, Grade V), aluminum hydroxide hydrate and, urethane and
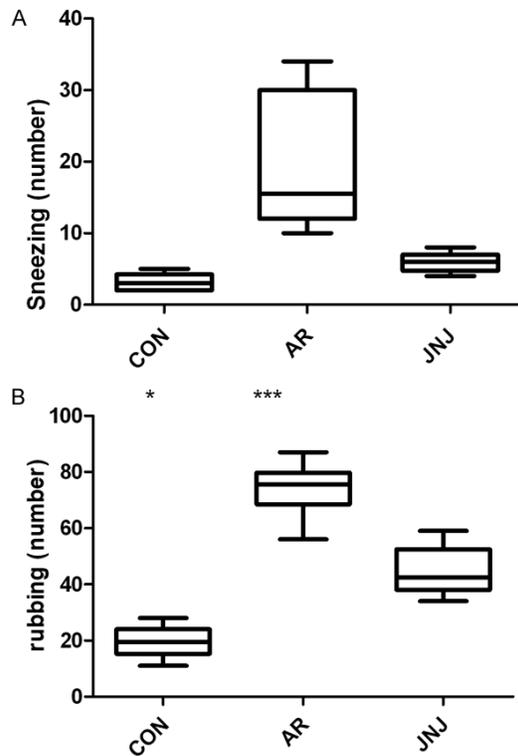
A

B

Figure 1. Comparison of nasal symptoms nasal sneezing (A) and nasal rubbing (B) among control group (CON) and allergic rhinitis group (AR) and JNJ7777120 group (JNJ). ***P<0.05, AR vs CON; *P<0.05, JNJ vs AR.

JNJ7777120 (1-[(5-Chloro-1 H-indol-2-yl) carbonyl]-4-methyl-piperazine) were purchased from Sigma-Aldrich (St. Louis, MO, USA). JNJ7777120, 1-[(5-Chloro-1H-indol-2-yl) carbonyl]-4-methyl-piperazine, was purchased from Sigma, USA (Lot J3770, Sigma).

Animals

Specific pathogen-free (SPF) male Wistar rats were purchased from Huafukang Biotech (Beijing, China). Animals were initiated into experiments at the age of 7-9 week-old with the initial body weights ranged between 250-300 g. Animals were group-housed with temperature and humidity maintained (20-26°C, 40-70% RH) and a light/dark cycle (12 h/12 h). Standard rodent's diet and water were given ad libitum. Conduction of the study complied with the Guide for Care and Use of Laboratory Animals of Jilin University.

Sensitization and antigen challenge

SPF male Wistar rats were acclimated for 1 week prior to the sensitization and antigen challenge followed. Animals were randomly divided into 3 groups (n=10 in each group) and treated as followed: (i) Normal group as control group (CON): rats were treated with saline, (ii) AR group (AR): OVA-sensitized/challenged group rat were sensitized and challenged with OVA. OVA formulation was prepared by emulsification in saline at the concentration of 100 mg/ml. 0.5 ml OVA (100 mg/ml) was administered on Days 0 and 7 by i.p. together with 0.5 ml of aluminum hydroxide (40 mg/ml) in saline. Day 21 after the last sensitization, rat were exposed to OVA drip (5% w/v diluted in sterile saline 25 µl administrated once a day in both nostrils for 1 week. From the fourth week, 5% OVA drip diluted in saline were given twice a week until the end of 12th week. (iii) JNJ-7777120 treated group (JNJ): rats were sensitized and challenged as in allergic rhinitis rat, and were given by 10 mg/kg JNJ7777120 once a week by nasal drop before challenge and were administrated intraperitoneally (i.p.) with OVA and aluminum hydroxide hydrate respectively during sensitization phase.

At the end of the experimental study, the animals were anesthetized using chloral hydrate (0.3~0.35 g/Kg) 24 h after the last challenge. 5 ml blood each rat were quickly taken from the heart blood, then standing at 4°C 2 hours before centrifugation (1500 g, 15 min), the Eppendorf packaging, stored in -80°C refrigerator. The nasal mucosa membrane was excised from the turbinate and nasal septum. The samples were divided into two parts. One part was left for collected for histopathological examination. The other part was dissected and homogenized for subsequent analysis.

Evaluation of allergic symptoms induced after allergen challenge

The numbers of sneezing and nose-rubbing motions during the 10-min period after the last allergen challenge were recorded and compared between the experimental groups by observers blinded to the experimental groups [8].

Th1/Th2 cytokines in nasal mucosal tissue

Preparation of nasal mucosa tissue homogenate: The tissue samples of nasal mucosa were weighted and homogenized (1:10, w/v) in 0.1 n 0.1 M phosphate buffer (pH 7.4) in an ice bath. The homogenate was centrifuged at 20000 g
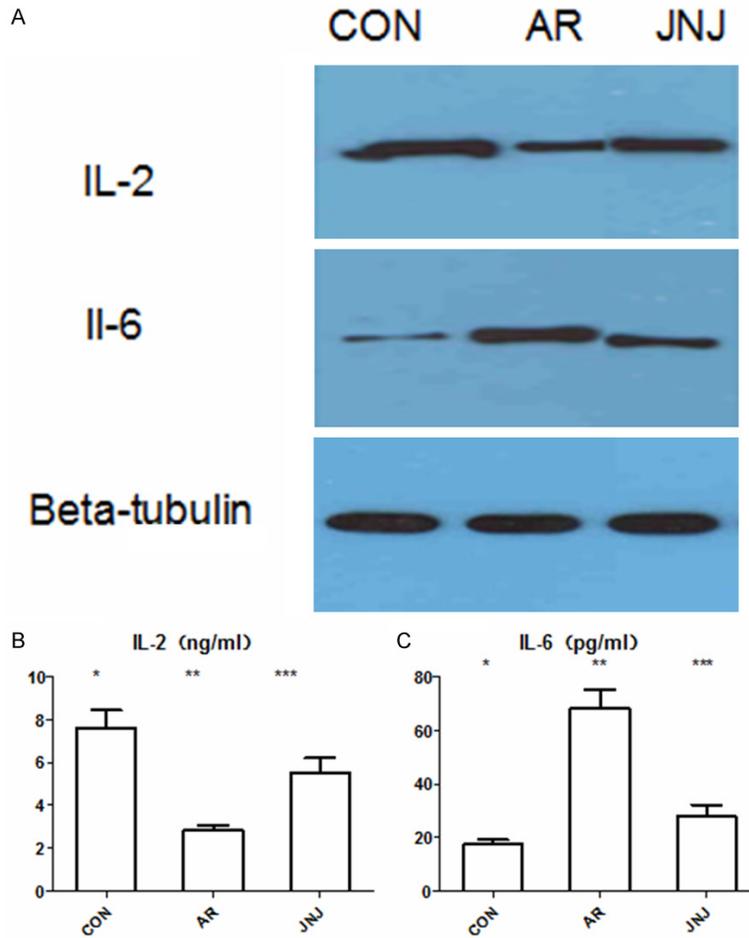
Figure 2. Comparison of expression of IL-2 and IL-6 in nasal mucosa by Western blotting (A) and in serum by ELISA (B and C) among control group (CON) and allergic rhinitis group (AR) and JNJ7777120 group (JNJ). *P<0.05, JNJ vs CON; **P<0.05, JNJ vs AR; ***P<0.05, JNJ vs CON.

eosin (H&E) stains were performed for general morphology and assessment of subepithelial inflammatory mediators and intraepithelial eosinophils, periodic acid Schiff (PAS) stain for identification of goblet cells in the epithelium. To quantify the goblet cell hyperplasia, ratio of goblet cell used a quantitative data of goblet cell hyperplasia in each rat were calculated. Masson's trichrome (MT) stain for extracellular matrix deposition (ECM) in nasal mucosa. The areas of ECM beneath the epithelial basement membrane in the MT stained tissue were termed of percentage. All histological examination was carried out in double blind manner.

Statistical analysis

The Graphpad software (GraphPad Software Inc. V5.01, San Diego, CA, USA) was applied in the study and the significance level was set at P<0.05. The number of sneezing and nasal rubbing motion were expressed as median and interquartile range and analyzed by Statistical analyses for non-parametric data were done using the Kruskal-Wallis test followed by Dunn's post-test correction. The expression of IL-2 and IL-6 were expressed as the average ± standard deviation and analyzed by unpaired t test with two-tailed Welch's correction. The number of eosinophil and globet cells per high-power field and the percentage of ECM were indicated as median and interquartile and analyses by Krukal-Wallis test.

for 30 min at 4°C. The supernatant was used for measurement of IL-2 and IL-6.

Western blotting: Western blotting analysis was used to detect the relative protein expression patterns of cytokines (IL-2, IL-6) in nasal mucosa of the CON, AR and JNJ groups. The results were based on a grade of grayscale (Santa Cruz Biotechnology, Dallas, TX, USA). ELISA was used to measure the protein level of IL-2 and IL-6 in serum.

Tissue histopathology

For each rat, the left nostril were removed and fixed in 4% paraformaldehyde and then embedded in paraffin. At that time, the left nostril of each rat was equally sectioned into three pieces from the apex of the lung. Hematoxylin and

Results

JNJ7777120 inhibit the OVA-induced allergic symptoms (sneezing, nasal rubbing)

We investigated the number of sneezing and nose-rubbing motions during the 10 minutes period after the last allergen challenge. The
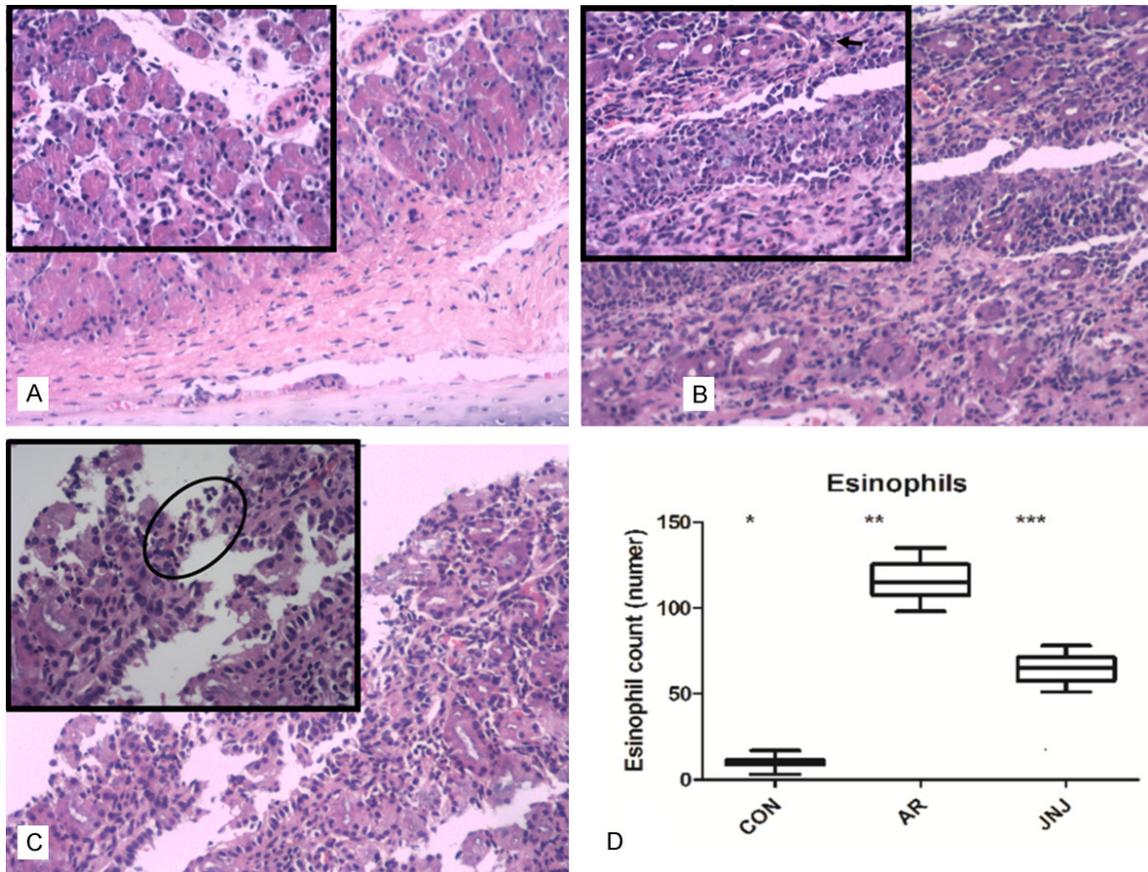
Figure 3. comparison of eosinophils Infiltration. (Hematoxylin and eosin staining, original magnification, ×50 and ×200). A: There was few esinophils in control group. B: There were some esinophils (the black arrow) in the nasal mucosa of JNJ7777120 treatment group. C: There were many esionophils infiltration in the nasal mucosa in allergic rhinitis group (the circle area). D: The eosinophil count was significantly higher in the AR group than in the JNJ7777120 group. The abbreviations as in **Figure 1**. Box and whiskers plot show median and interquartile range. *P<0.05, JNJ vs AR; **P<0.05, JNJ vs CON, ***P<0.05, JNJ vs CON according to Kruskal-Wallis tests.

number of sneezing motion was 3 [2, 4.25] in the control group, 15.5 [12, 30] in the AR group, and 6 [4.75, 7] in the JNJ7777120 group. The number of nasal rubbing motion was 19.5 [15.25, 24] in the control group, 75.5 [68.5, 79.75] in the AR group, and 42.5 [38, 52.5] in the JNJ7777120 group. The number of sneezing motion was significantly lower in the control group than in the AR group (P<0.05, **Figure 1A**), whereas the number of sneezing motion was significantly higher in the AR group than in the JNJ7777120 group (P<0.05, **Figure 1A**). The number of nasal rubbing motion was also lower in the control group than in the AR group (P<0.05, **Figure 1B**), whereas it was higher in the AR group than in the JNJ7777120 group (P<0.05, **Figure 1B**).

JNJ7777120 decreased IL-6 and increase IL-2 expression in nasal mucosa and serum

Expression of IL-2 and IL-6 were measured as by western blotting in nasal mucosa **Figure 2A**. There were significant differences in the expression of IL-2 and IL-6 in nasal mucosa among the control and AR and JNJ7777120 group. JNJ7777120 could inhibit the Th1/Th2 reaction induced by AR. The expression of IL-2 in serum was 7.58±0.38 ng/ml in the control group, 2.84±0.93 ng/ml in the AR group, 5.52±0.31 ng/ml in the JNJ7777120 group. The IL-2 expression was significantly lower in the AR group than in the JNJ7777120 group (t=8.271, P=0.0006) and control group (t=12.08, P=0.0003) (**Figure 2B**), while JNJ7777120
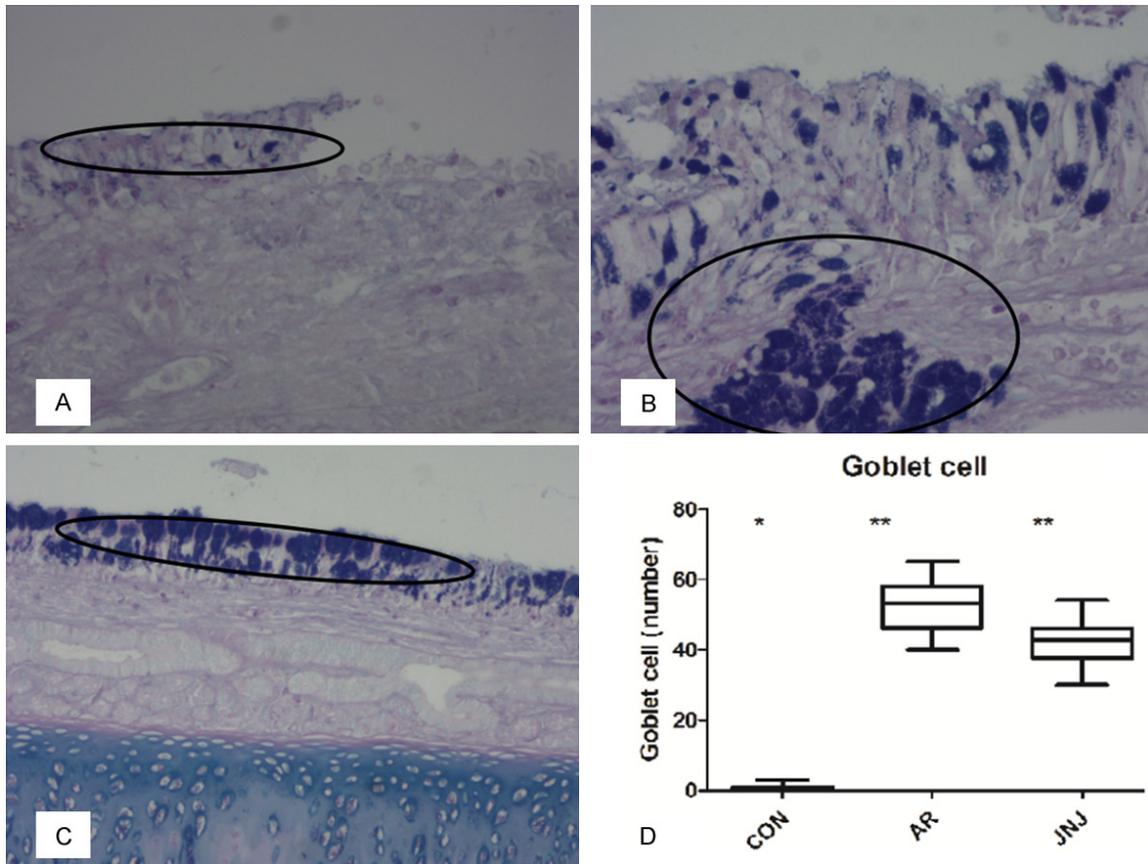
Figure 4. Goblet cells in nasal epithelium. (alcian blue-periodic acid-Schiff, AB-PAS; original magnification, ×200). Black circle area indicates infiltration of goblet cell. A: There were few goblet cells in the normal control group. B: There was almost all goblet cells in allergic rhinitis group. C: There were some goblet cells in JNJ7777120 treatment group. D: The count of goblet cells is significantly lower in treatment group than that of AR group. Box and whiskers plot show median and interquartile range. *P<0.05, JNJ vs AR; **P<0.05, JNJ vs CON, ***P<0.05, JNJ vs CON according to Kruskal-Wallis tests. The abbreviations as in **Figure 1**.

group lower than control group (t=4.289, P=0.0004). The expression of IL-6 in serum was 17.36±0.81 pg/ml in the control group, 68.12±0.3.17 pg/ml in the AR group, 27.76±1.91 pg/ml in the JNJ7777120 group. The IL-6 expression was significantly higher in the AR group than in the JNJ7777120 group (t=10.90, P<0.0001) and control group (t=15.50, P=0.0001), while JNJ7777120 group higher than control group (t=5.016, P=0.004) (**Figure 2C**).

Effect on development of nasal mucosa inflammation and remodeling

JNJ7777120 could significant alleviate the histological changes originated from AR, such as eosinophils infiltration, goblet cell hyperplasia, epithelial damage and deposition of extracellular matrix in the nasal mucosa.

JNJ7777120 decrease the eosinophil infiltration: H&E staining was used to visualize eosinophils. Eosinophils were counted in 5 different areas with a constant mucosal length on slides under a high-power field (×200) of 5 in each group. **Figure 3** shows eosinophil infiltration in the lamina propria and the number of eosinophil. The number of eosinophils were 10/2,500 μm^2 [8.5, 11.5] in the control group, 115/2,500 μm^2 [107.5, 125.5] in the AR group, 65/2,500 μm^2 [57.5, 71.25] in the JNJ7777120 group. The eosinophil count was significantly higher in the AR group than in the JNJ7777120 group and control group (both P<0.05), while JNJ7777120 group higher than control group (P<0.05).

JNJ7777120 inhibit the goblet cell hyperplasia: AB-PAS staining demonstrated the number of goblet cells in the epithelium. Goblet cells were
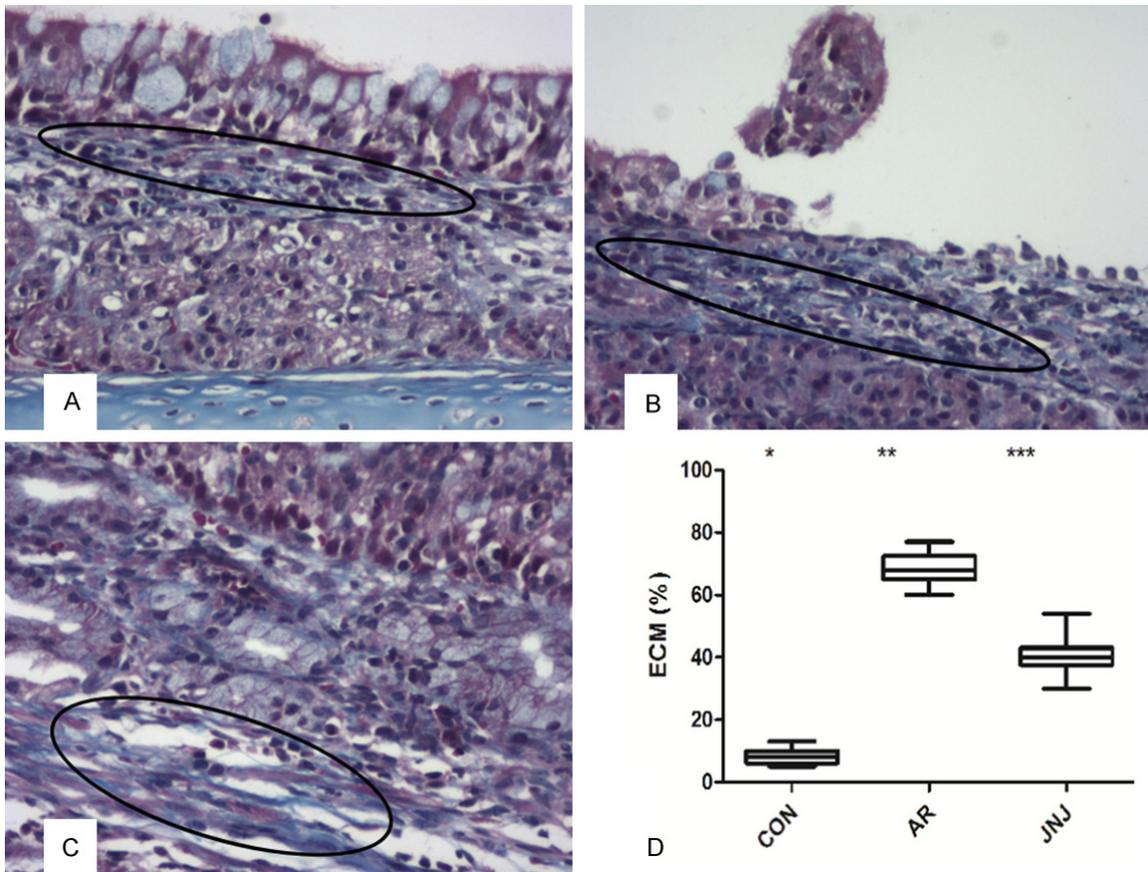
Figure 5. Extracellular matrix deposition of nasal mucosa. Collagen deposition was blue stained around the peripheral blood vessels and subepithelial glands (circle area, MT staining ×200). A: There was less than 8% ECM in normal control group. B: There was almost 60% ECM in allergic rhinitis. C: There was nearly 40% ECM in the JNJ7777120 treatment group. D: The percentage of ECM was significantly higher in the AR group than in the control and JNJ7777120 group. The abbreviations as in **Figure 1**. Box and whiskers plot show median and interquartile range. *P<0.05, JNJ vs AR; **P<0.05, JNJ vs CON, ***P<0.05, JNJ vs CON according to Kruskal-Wallis tests. The abbreviations as in **Figure 1**.

counted in 5 different areas within a constant mucosal length on slides under a high-power field (×200) of 5 in each group. **Figure 4** shows goblet cell nasal epithelium and the number. The number of goblet cells were 0/2,500 µm² [0, 1] in the control group, 53/2,500 µm² [46.5, 58] in the AR group, 43/2,500 µm² [37.5, 46] in the JNJ7777120 group. The goblet cell count was significantly higher in the AR group than in the JNJ7777120 group and control group (both P<0.05), while JNJ7777120 group higher than control group (P<0.05).

JNJ7777120 inhibit the extracellular matrix deposition: Collagen was stained blue, deposited in subepithelial glands, and blood vessels around. In the saline control group, there was little collagen (MT staining) beneath the base-

ment membrane of the epithelium and around the glands and vasculature (**Figure 5**). The percentage of ECM were 8% [6, 10] in the control group, 68% [65, 72.5] in the AR group, 40% [37.5, 43] in the JNJ7777120 group. In the AR group, there was a significant increase in ECM deposition in the submucosa of the nasal mucosa (P<0.05). This change was significantly inhibited by administration of JNJ7777120 (P<0.05).

Discussion

HR4 has been recently advocated as drug target for of eosinophil inflammatory disorders [9], while it was reported that JNJ7777120, the specific chemical inhibitor, could decrease the allergic symptoms and change some biochemi-

cal reaction in asthma [10] and AR [11]. Our study found that JNJ7777120 could weaken or delay remodeling in AR rat model stimulated by OVA compared with the AR group.

In our study, we found that JNJ7777120 could decreased all the remodeling process in AR, including the eosinophils accumulation, the goblet cell hyperplasia, epithelial damage and also the deposition of extracellular matrix [12, 13]. It was known that eosinophil migration from the bloodstream into the sites of inflammation was mediated by the leukocyte trafficking and pro-inflammatory responses by HR4 [14-17]. As JNJ7777120, some other small-molecule antagonists of the H4 receptor inhibit eosinophil migration [18-20] too. Goblet cell metaplasia attenuated allergic responses by inducing acute plasma leakage and edema by histamine and its receptors [21], our study tells that HR4 may involve the allergic reaction as HR1 too. We also found that JNJ7777120 was also found negative function in migration of nasal fibroblasts and ECM deposition as other reports [22-24].

Our study also indicated that HR4 may involve in remodeling by changing Th1/Th2 inflammation balance through the evidence that nasal mucosa and serum inflammation marker change developing together with tissue structure remodeling. IL-2 was acted as the biomarker of Th1 cell reaction while IL-6 as the biomarker of Th2 cell reaction in our study, because both of them also might play an important role in remodeling such as eosinophils accumulation and fibrosis. For example, in allergic response, IL-2 acts as a negative autocrine regulator of EOS migration [25]. IL-6 also has an effect on the production of collagens, tissue inhibitor of metalloproteinases (TIMPs), and glycosaminoglycan in fibrogenesis [26]. H4R-induced IL-6 production was mediated by PI3K, ERK, JNK and p38 pathways [27]. It was already reported that in the JNJ could dismiss existing inflammation the asthma by modulation of Th2 cytokines such as IL-13 and IL-5 [28]. Our study found that in allergic rhinitis animal model, JNJ777710 could also functioned similarly by changing the unbalance by Th1/Th2 response.

In conclusion, histamine acting through the H4R provides an important link between the Th1/Th2 inflammation and tissue remodeling. The combined immunosuppressive and anti-inflammatory effects of H4R antagonist JNJ7777120 indicated that the HR4 is an extremely attractive target for the treatment of a variety of diseases. In other words, our results suggest that JNJ7777120, H4R antagonists may have potential benefits for the treatment of allergic rhinitis by the inhibiting the development of nasal remodeling and change Th1/Th2 unbalance.

Disclosure of conflict of interest

None.

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