Original Research

JCMSG

Mechanisms of action of topical budesonide and oral prednisolone in a rat model of chronic rhinosinusitis

YunCheng Bai[2], Yu Ding[3], Wenjing Ding[4]， Li Kang[4], Xi Zhang[4] ,Yan Lin[1,*]

Abstract

The inflammation and oxidase have emerged as two pivotal events in chronic rhinosinusitis (CRS) progression. Topical budesonide and oral prednisolone have been extensively used in the therapy of CRS. However, the difference of treatment effect of budesonide and prednisolone on CRS still unclear. This study found that inflammation level was upregulated in CRS rats compared with sham rats. Topical budesonide and oral prednisolone both suppressed the secretion of inflammation in serum of CRS rats, and there was no significant difference between two therapy approaches. Moreover, treatment with budesonide and prednisolone both attenuated the histopathological injury of nose mucous membranes of CRS rats. Furthermore, the expressions of anti-oxidation protein were detected by IHC, qRT-PCR and western blot, the results demonstrated that the expression of anti-oxidation proteins were upregulated in CRS rats compared with sham rats, and the treatment with budesonide and prednisolone both attenuated the expression of anti-oxidation proteins. Finally, we shown that budesonide and prednisolone inhibited the progression of CRS in rats by inhibiting TLR4/TRAF6/NF-κB signaling pathways. Collectively, these findings indicated that tipcal budesonide and oral prednisolone could inhibited the progression of CRS by blocking TLR4/TRAF6/NF-κB signaling pathways.

Keywords: chronic rhinosinusitis, budesonide, prednisolone, NF-κB

1. Department of Head and Neck Surgery, The First Affiliated Hospital of Kunming Medical University, No. 295 Xichang Road, Kunming, 650032, Yunnan, China

2. Orthopaedics, First People's Hospital of Yunnan Province, No. 157, Jinbi Road, Kunming, 650034, Yunnan, China

3. The second Affiliated Hospital of Kunming Medical University, No. 374 Dianmian Road, Kunming, 650033, Yunnan, China

4. Kunming Medical University, No. 1168, Chunrong West Road, Kunming, 650500, Yunnan, China

*Correspondence: linyan20072021@163.com

Introduction

Rhinosinusitis, also known as Sinusitis, is a heterogenous inflammatory disease, which involving the nose mucous membranes and paranasal sinuses. It is a common disease with a prevalence rate ranging from 4.5 to 12%[1], and smokers are more common than non-smokers[2]. Rhinosinusitis is defined as acute rhinosinusitis (ARS) if it lasts less than 4 weeks, and as chronic rhinosinusitis (CRS) if it lasts for longer than 12 weeks[3]. The diagnostic symptoms of CRS include nasal congestion, nasal discharge, facial pain, and reduction in smell[3, 4].

The mainly treatment for CRS is nasal adrenocorticosteroids[5] and oral corticosteroid[6] to address the underlying inflammatory disorder of nasal mucosa or body, respectively[7]. Budesonide, commonly known by the trade name Pulmicort, is a medication of the corticosteroid type[8]. Budesonide is available as an inhaler[9], nebulization solution[10], pill[11], nasal spray[12], and rectal forms. Budesonide was approved in April 2021 by the UK's NHS to treat COVID-19 on a case-by-case basis[13]. Moreover, the nasal spray of budesonide is used for allergic rhinitis[12], CRS[14] and nasal polyps[15], it's common side effects include respiratory infections, cough, and headaches[16]. Prednisolone is a corticosteroid drug with predominant glucocorticoid and low mineralocorticoid activity[17], it is an oral glucocorticosteroid that is usually used to suppress the immune system and reduce inflammation in conditions such as asthma[18], CRS[19], chronic obstructive pulmonary disease[20], and rheumatism[21]. Because CRS is a chronic disease, there are concerns about the long-term use of systemic medications. Long-term use of medications may lead to adverse effects, drug interactions, and antimicrobial resistance[22]. Therefore, the choice of medication and treatment way that can have a profound impact on patients' quality of life and health care expenditures. In this study we were interested in studying the efficiency of topical budesonide and oral prednisolone in a rat model of CRS.

Material and methods

Animal

Eighteen 10-12-week-old Sprague-Dawley (SD) rats, with no evidence of upper respiratory tract infection were purchased from Kunming Medical University (Kunming, China). All experiments were approved by the Kunming Medical University Animal Care Committee and conducted in accordance with the Ethical Guidelines for the Care and Use of Animal for Research Purposes.

Fifteen rats were used to construct chronic rhinosinusitis model. Briefly, the long hair on the back of the rat's nose was shaved off, the skin was cut along the septum, the periosteum from the nasal septum to the medial canthus was separated under aseptic conditions, a hole with a diameter of about 1.5 mm was drilled at the level of the medial canthus and at a distance of 2 mm on the right side of the nasal septum with a dental drill, a gelatin sponge was inserted into the drilled maxillary sinus with ophthalmic forceps, 0.1 ml of 10^9 cfu/ml of *Staphylococcus aureus* was dropped, and the periosteum and the skin were sutured. The rest three rats were as sham group, the long hair on the back of the rat's nose was shaved off, the skin was cut along the septum, and sutured the skin, and the rats were fed for 8 weeks. After

successful model establishment, the chronic rhinosinusitis rats were divided into five groups, namely, Model group, vehicle 1 group, Budesonide group, vehicle 2 group, and Prednisolone group. The rats of sham group and the model group were not treated. Budesonide (Tianjin pacific chemical & pharmaceuticao CO., LTO, China) was spraying into nasal (Solarbio, China) cavity of rats of budesonide group, 50 μl (1.28μg/ μl) on each side, once a day for 14 days. Saline was spraying into nasal cavity of rats of vehicle 1 group, 50 μl on each side, once a day for 14 days. Rats in prednisolone group were fed a daily dose of 1 mg/kg of prednisolone (Tianjin pacific chemical & pharmaceuticao CO., LTO, China) dissolved in saline for 14 days. Rats in vehicle 2 group were fed an equivalent volume of saline daily for 14 days.

Enzyme-linked Immunosorbent Assay (ELISA)

The ELISA was performed to detect the contents of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in serum of rats. Blood (1 mL) were extracted from the vein blood, and the obtained the serum. IL-1β, IL-6 and TNF-α content were detected using IL-1β ELISA kit, IL-6 ELISA kit and TNF-α ELISA kit (Bio-Technology Co., Ltd. XinBoSheng) strictly according to the manufacturer's instruction.

Histological assessment (H&E)

Samples of mucous membranes were harvested from rat under general anesthesia. H&E staining was performed using a Hematoxylin and Eosin Staining Kit (Beyotime, China). The harvested tissues were fixed in 10% formaldehyde for 1 day, followed by dehydration, permeabilization, wax dipping, paraffin embedding, and cutting into 3 μm sections. The sections were staining with hematoxylin for 5 min, followed by stained with eosin solution for 30 sec. After that, the sections were dehydrated and mounted using neutral gum. The pictures of histologic changes were observed and captured by microscopy (Leica, German).

Immunohistochemistry (IHC)

Immunohistochemistry was performed to detected the distribution and expression of Lysozyme, Eosinophils, TNFα, PLUNC and Cu/ZnSOD, and the IHC was performed as described previously. Briefly, the mucous membranes tissues of rats were fixed in 10% formaldehyde for 1 day, followed by dehydration, permeabilization, was dipping, paraffin embedding, and cutting into 4 μm sections. After dewaxing, the endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol solution, and the antigen was repaired with 0.3% Tris-EDTA buffer (pH 9.0), then blocked with 3% BSA for 30 min. After that, the sections were incubated with primary antibodies Lysozyme (abcam, 1:1000, China), Eosinophils (abcam, 1:1000, China), TNFα (abcam, 1:100, China), PLUNC (bioss, 1:1000, China) and Cu/ZnSOD (abcam, 1:4000, China). After that the sections were incubated with Secondary antibody coupled with horseradish peroxidase for 30 min, the nuclei were counterstained with hematoxylin solution. The staining intensity was assessed by IHC profiler plug-in image J software. Intensity ratings were as follows: strongly positive, positive, weakly positive, and negative expression.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR assay was conducted to detected the mRNA expression level of TNFα
PLUNC, Cu/ZnSOD and DJ-1. Total RNA of mucous membranes of rats were extracted based on the Trizol method (Invitrogen Inc., China). Reverse transcription reagents

(Applied Biosystems, China) were used for reverse transcription of mRNA to synthesize cDNA. The cDNA was supplemented with SYBR Select Master Mix (Thermo Fisher Scientific, China) strictly follow the manufacturer's instruction. RT-q PCR was performed using an ABI ABI 7500® real-time PCR system (Applied Biosystems, China) to detect the expression of mRNA. The following primers were provided by by BioTNT (BioTNT, Shanghai, China): TNFα forward: 5′-CTT CAG GGA TAT GTG ATG GAC TC-3′, reverse: 5′-GGA GAC CTC TGG GGA GAT GT-3′; PLUNC forward: 5′-ATA AGA ATG CGG CCG CCT AAG AGC AAA GAT GTT TC-3′, reverse: 5′-ATA AGA ATG CGG CCG CAC CTT GAT GAC AAA CTG-3′; Cu/ZnSOD forward: 5′-TTC GAG CAG AAG GCA AGC GGT GAA-3′, reverse: 5′-AAT CCC AAT CAC ACC ACA AGC CAA-3′; DJ-1 forward: 5′-ACT GCT CTA GTC CTG TGG GT-3′, reverse: 5′-CAG CTC GCC TCA TGA CAT CT-3′; GAPDH: forward: GCA TGG ACT GTG GTC ATG AG; reverse: TGC ACC ACC AAC TGT GGT CAT GAG. PCR was performed for 40 cycles, and the mRNA expression of the target genes were analyzed relative to the mRNA expression levels of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta Ct}$ method.

Western blot

Western blot assay was performed to detected the protein expression levels of TLR4, TRAF6, NFκB/P65, Lysozyme, PLUNC, Cu/ZnSOD. The proteins were extracted from mucous membranes of rats, and the protein concentrate of target proteins were detected by bicinchoninic acid (BCA) protein quantification kit (BCA1-1KT; Sigma–Aldrich Chemical Company) strictly follow the instructions and adjust the protein concentration to the same amount as the loading buffer with deionized water. Proteins were separated on a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred to polyvinylidene difluoride (PVDF) membranes. After that, the membranes were blocked with 5% non-fat milk for 1 h at room temperature. Follow the washed three times with PBS, the membranes were incubated with primary antibodies TLR4 (1: 500, abcam, China), TRAF6 (1: 2000, abcam, China), NFκB/P65 (1: 1000, abcam, China), Lysozyme (1: 10000, abcam, China), PLUNC (1: 10000, abcam, China), Cu/ZnSOD (1: 50000, abcam, China) and β-actin (zsjqbio, 1:2000, China) overnight at 4 ℃. Following this, the membranes were rinsed in TBST supplemented with a horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at 37 ℃. After

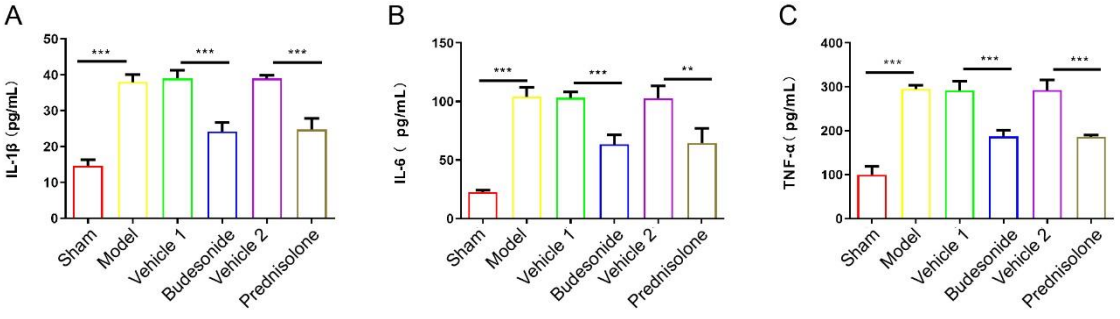

Figure 1 The effect of topical budesonide and oral prednisolone on inflammation in the serum of CRS rats. (A) The secretion of IL-1β in serum of rats were detected by ELISA. (B) The secretion of IL-6 in serum of rats were detected by ELISA. (C) The secretion of TNF-α in serum of rats were detected by ELISA.

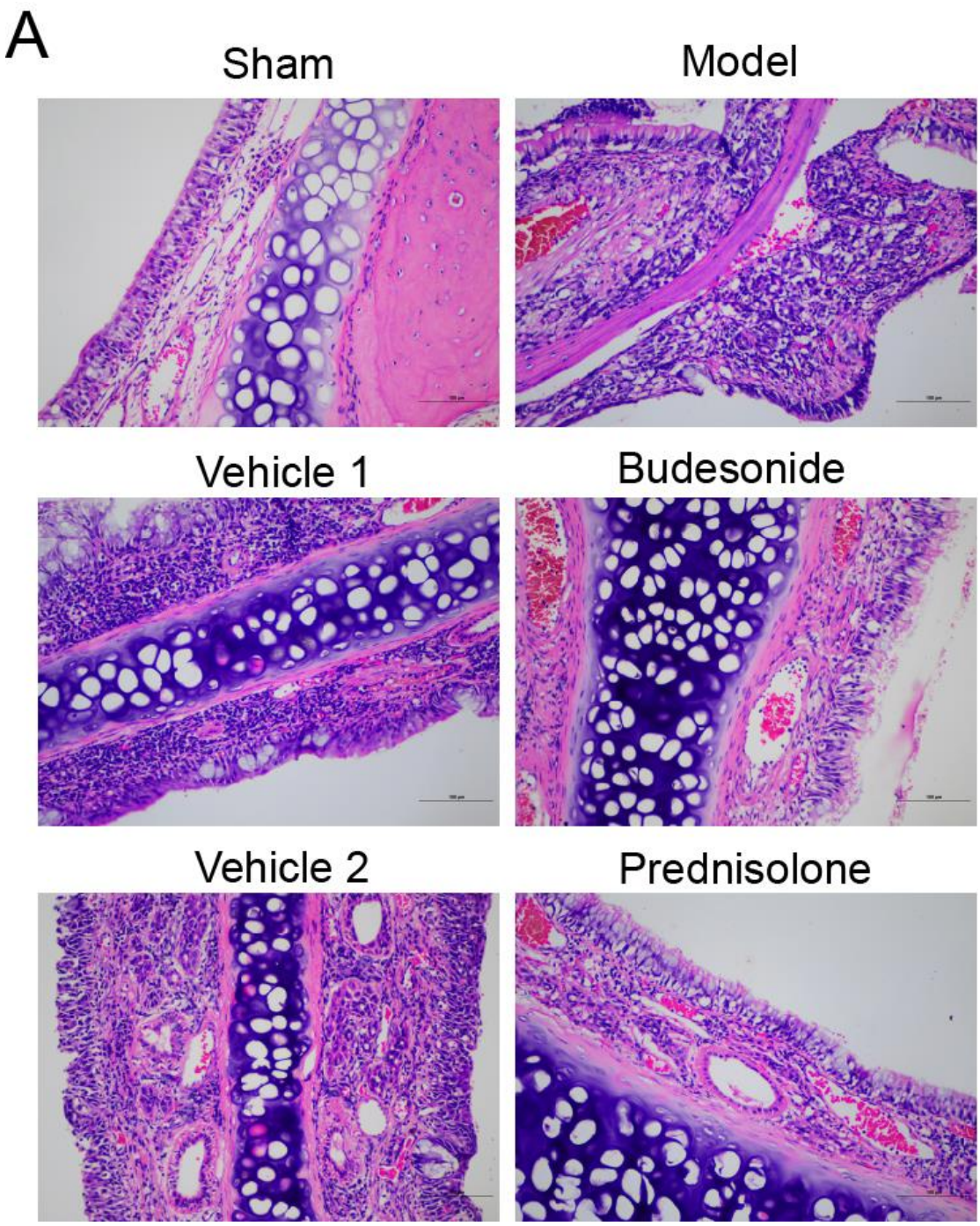

Figure 2 Topical budesonide and oral prednisolone attenuated the histopathological injury of nose mucous membranes of CRS rats (400 ×). The results of HE staining exhibited that nasal mucosa injury was observed in CRS rats, and the treatment with budesonide and prednisolone both attenuated this injury of nasal mucosa.

rinsing the membranes in TBST, the enhanced chemiluminescence (ECL) reagents (Sigma–Aldrich Chemical Company, China) were used to visualize the protein. The β-actin used as loading control, the protein levels were illustrated by the ratio of the grey value of the target band and the loading control band using Image J (Media Cybernetics, San Diego, California USA).

Statistical analysis

All statistical analysis were performed by the Graphpad 8.0 software, and consistent with the normal distribution and the homogeneity

variance test. Data were presented as mean ± standard deviation (SD), the difference among groups were compared by one-way analysis of variance (ANOVA). A *P*-value < 0.05 was considered to be statistically significant.

Results

Budesonide and prednisolone treatment decreased the secretion of inflammatory factor in ARS

The contents of inflammatory factors in serum of rats of each group were detected using ELISA kit so as to show the effect of budesonide and prednisolone on inflammation. The results shown that the levels of IL-1β, IL-6, and TNFα was increased in chronic rhinosinusitis rats compared with sham rats (Figure 1.A-C). The levels of IL-1β, IL-6, and TNFα were decreased by the topical treatment with budesonide and oral prednisolone (Figure 1.A-C). There was no noteworthy difference between budesonide group and prednisolone group (Figure 1.A-C). Meanwhile, H&E staining was performed to determine the histopathological conditions of nose mucous membranes of rats. The results demonstrated that the mucosal epithelium was disorderly aligned, hyperplasia of goblet cells, sub-mucosal gland and fiber tissues was occurred in nose mucous membranes of chronic rhinosinusitis rats compared with sham rats (Figure 2). However, the aforesaid results were improved after the treatment of budesonide and prednisolone, and there was no significance difference between budesonide group and prednisolone group (Figure 2). These results demonstrated that budesonide and predniso-

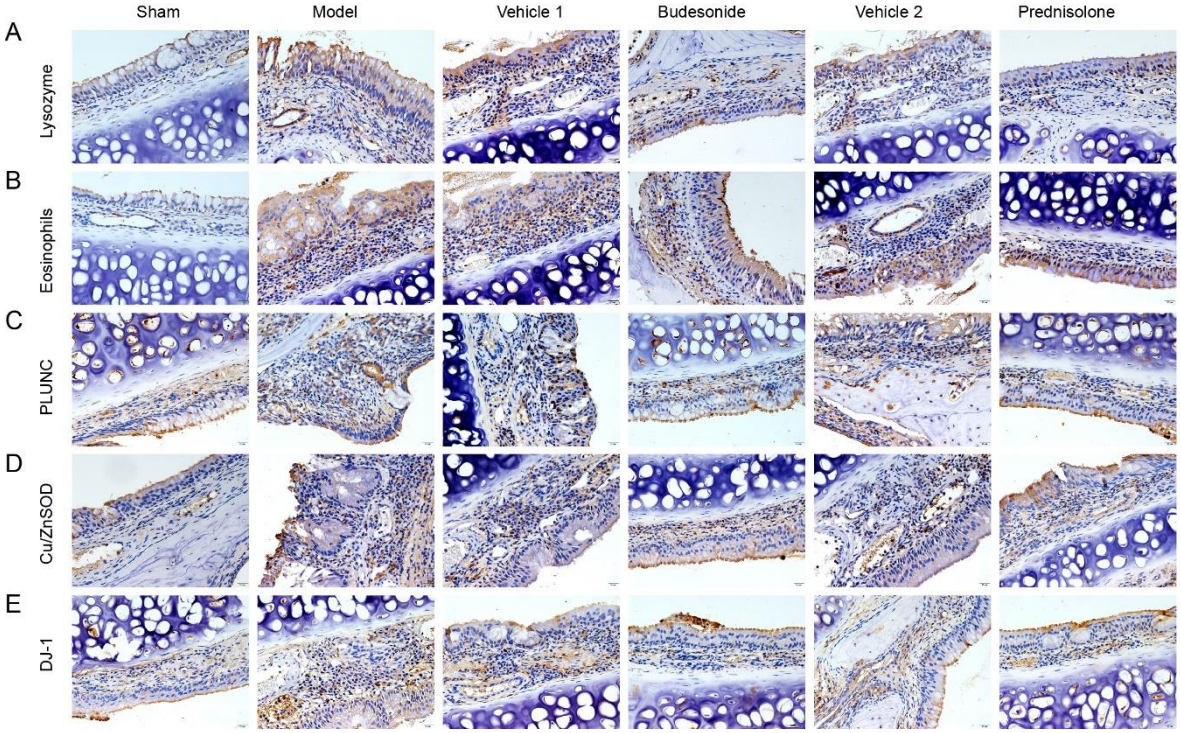

Figure 3 The effect of topical budesonide and oral prednisolone attenuated the inflammation of nose mucous membranes of CRS rats (400 ×). (A). The expression intensity and location of Lysozyme detected by IHC staining. (B). The expression intensity and location of Eosinophils detected by IHC staining. (C). The expression intensity and location of PLUNC detected by IHC staining. (D). The expression intensity and location of Cu/ZnSOD detected by IHC staining. (E). The expression intensity and location of DJ-1 detected by IHC staining.

lone may sever as anti-inflammatory protection factor in chronic rhinosinusitis, and there was no significance difference between topical treatment with budesonide and oral prednisolone.

Budesonide and prednisolone treatment decreased ROS

Previous studies demonstrated that oxidative stress induced by both environmental and intrinsic stimuli underlies the onset and persistency of chronic rhinosinusitis, which lead to epithelial damage, accelerated mucosal inflammation, and prolonged eosinophilic inflammation. Therefore, IHC was performed to detect the expression of the anti-oxidation protein and immunoreactive protein of nasal mucosa in differently treated mice, and the results were displayed in Figure 3 A-E. The results demonstrated that the expression intensity and location of Lysozyme, Eosinophils, TNFα, PLUNC and Cu/ZnSOD were consistent in same tissue. They are mainly expressed in mucosal epithelium and glandular epithelium, the intensity of positivity in chronic rhinosinusitis group, vehicle 1 group and vehicle 2 group were predominantly strongly positive (+++), negative (-) was mainly observed in sham group, and weakly positive (+) was mainly observed in budesonide and

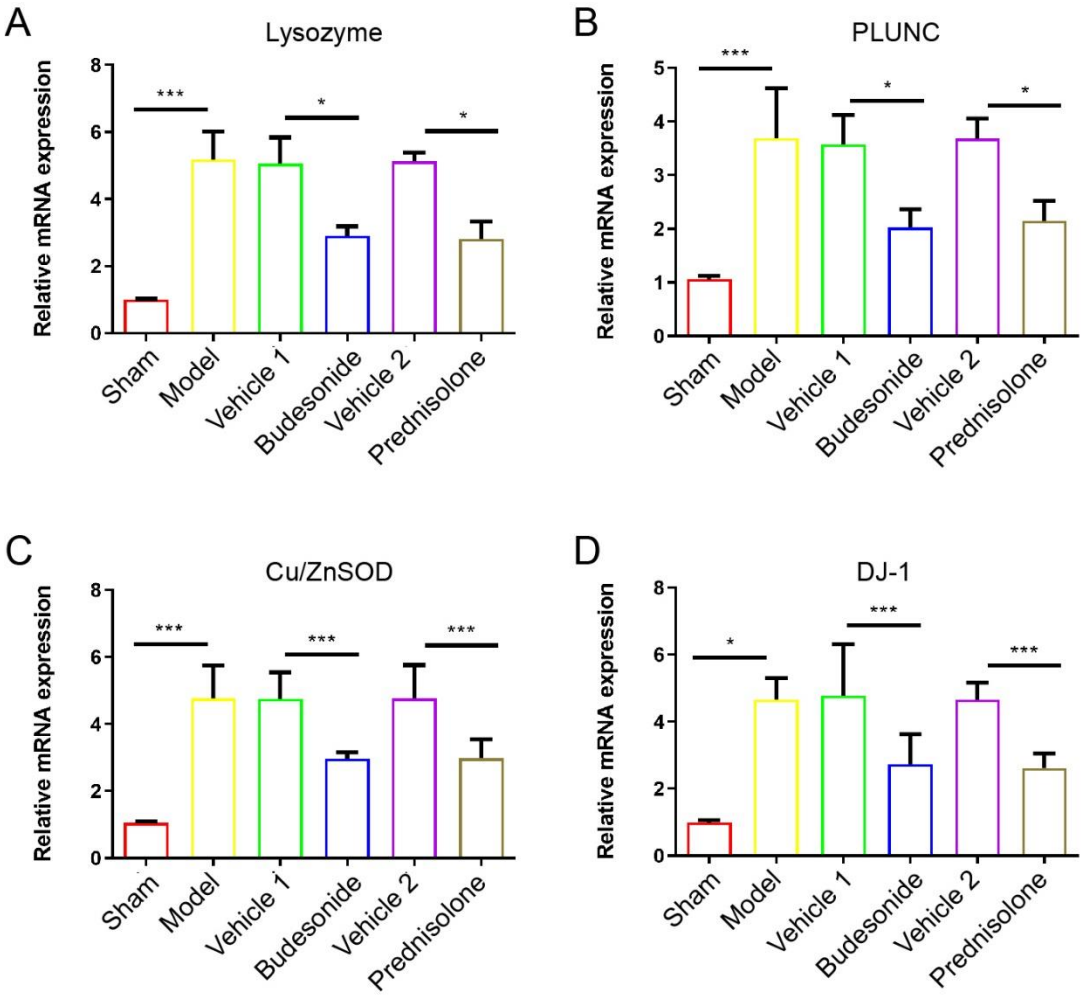

Figure 4 The effect of topical budesonide and oral prednisolone attenuated the inflammation of nose mucous membranes of CRS rats. (A). The mRNA expression of Lysozyme in nose mucous membranes detected by RT-qPCR. (B). The mRNA expression of PLUNC in nose mucous membranes detected by RT-qPCR. (C). The mRNA expression of Cu/ZnSOD in nose mucous membranes detected by RT-qPCR. (D). The mRNA expression of DJ-1 in nose mucous membranes detected by RT-qPCR.

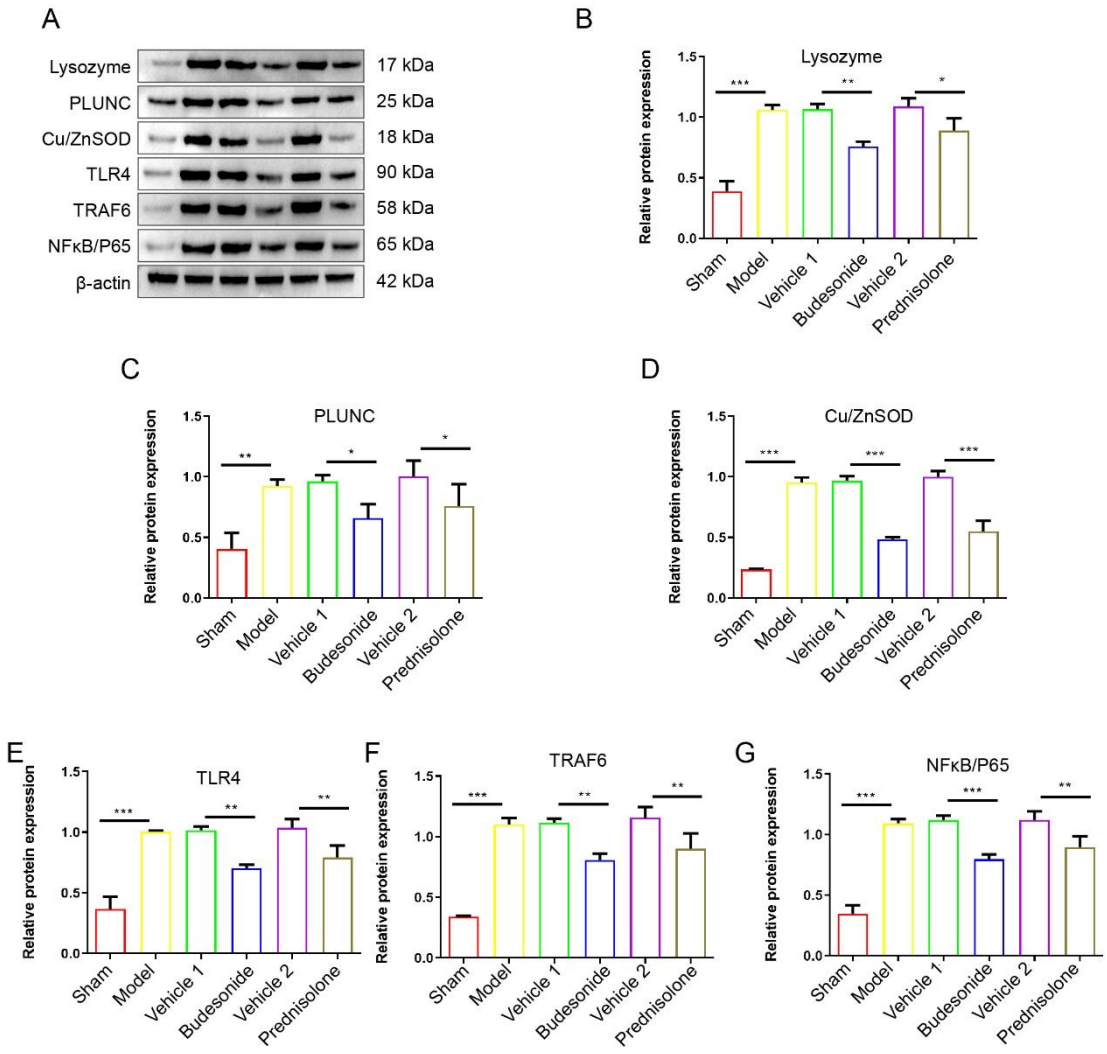

Figure 5 The effect of topical budesonide and oral prednisolone attenuated the inflammation of nose mucous membranes of CRS rats by TLR4/TRAF6/NF-κB signaling pathway. (A) Protein bands of anti-oxidation protein and TLR4/TRAF6/NF-κB signaling pathway relative protein in each group were detected by western blot assay. (B) Relative protein expression of Lysozyme. (C) Relative protein expression of PLUNC. (D) Relative protein expression of Cu/ZnSOD. (E) Relative protein expression of TLR4. (F) Relative protein expression of TRAF6. (G) Relative protein expression of NFκB/P65.

prednisolone treatment group. Furthermore, the mRNA expression of Lysozyme, TNFα, PLUNC and Cu/ZnSOD in masal mucosa were detected by RT-qPCR (Figure 4A-D). Compared with the sham group, the expression of Lysozyme, TNFα, PLUNC and Cu/ZnSOD in model group significantly increased, which attenuated by treatment with budesonide and prednisolone. The expression of Lysozyme, TNFα, PLUNC and Cu/ZnSOD in the model group, vehicle 1 group and vehicle 2 group were not dramatically different. These results indicated that the positive intensity of Lysozyme, Eosinophils, TNFα, PLUNC and Cu/ZnSOD were increased in CRS, but attenuated by topical treatment with budesonide and oral prednisolone. And topical treatment with budesonide and oral prednisolone shown same results.

Budesonide and prednisolone treatment relieved inflammatory phenotypes via TLR4/TRAF6/NF-κB signaling pathway

Evidences revealed that Toll-like receptor 4

(TLR4)/ TRAF6/NF-κB signaling pathway is essential for oxidative stress and inflammatory responses of ARS. To further explore the mechanisms underlying the anti-inflammatory roles of budesonide and prednisolone in ARS, the protein expression levels of TLR4, TRAF6, NFκB/P65, Lysozyme, PLUNC, Cu/ZnSOD were detected by western blot (Figure 5A). As shown in Figure 5B-D, the oxidative stress, and inflammatory responses relative protein Lysozyme, PLUNC and Cu/ZnSOD were remarkably increased in ARS rats compared with the sham mice, and there was no significantly difference between vehicle 1 group, vehicle 2 group and model group. Furthermore, the oxidative stress and inflammatory were no noteworthy between budesonide group and prednisolone group. To illustrated that whether TLR4/TRAF6/NF-κB signaling pathway regulated the oxidative stress and inflammatory responses of ARS, key molecular along the TLR4/TRAF6/NF-κB signaling pathway were detected in the nasal mucosa from different group by western blot. As show in Figure 5A and Figure 5E-G, the expression of TLR4 and its downstream molecule TRAF6 and NF-κB was upregulated in model group compared with sham group, while this tendency was attenuated by treatment with budesonide and prednisolone, respectively. There were no noteworthy differences between the budesonide group and prednisolone group. Taken together, these results suggest that topical budesonide and oral prednisolone inhibit the progression of ARS, while there is no significant difference in treatment with topical budesonide and oral prednisolone.

Discussion

The prevalence of chronic rhinosinusitis (CRS) is a heterogeneous disease characterized by inflammation of the nasal cavity and paranasal sinuses, which brings about many troubles to the daily life with more than 10% of adult population affected around the world[4]. There are a lot of therapy medicines for CRS, topical budesonide and oral prednisolone was effective treatment for CRS[7]. However, short-term use and long-term use of budesonide and prednisolone leaded to side effects, and the differences between topical budesonide and oral prednisolone of CRS rat model still unclear. This study is designed to investigate the therapeutic effect in topical budesonide and oral prednisolone of CRS rat model.

In this study, the inflammatory factors in serum of CRS rats were significantly increased compared with sham rats, suggested that the secretion of inflammatory factor was increased in CRS. It has been reported that CRS is an inflammatory disease[23], and topical budesonide[24, 25] and oral prednisolone[5] suppressed the progression of CRS via reduce the inflammation in CRS. Obviously, treatment of budesonide and prednisolone decreased the secretion of inflammatory factor in CRS rats, respectively. While there was no significantly difference between treatment with budesonide and prednisolone. The inflammatory mechanism of CRS refers to the molecular pathways leading to mucosal inflammation and tissue remodeling, and mucosal inflammation and tissue remodeling are the broad features of CRS[26]. In the present study, the histopathological injury of nose mucous membranes was increased in CRS rats, while treatment of budesonide and prednisolone both attenuated the histopathological injury.

Recently studies have suggested that oxidative stress was associated with the pathogenesis of CRS[27]. The secretion of pro-inflammation lead to oxidative stress, which causes symptoms such as nasal obstruction, increased nasal discharge and dry mucous membranes[28]. Oxidative stress is a common pathway

involved in cellular dysfunction, tissue injury, and organ failure[29], oxidative stress occurs when the formation of ROS exceeds the body's ability to metabolize them or when the antioxidant defence mechanisms are depleted[30]. Antioxidant systems can stabilize free radicals, consequently reducing the oxidative stress[31]. Our results demonstrated the mRNA expression and protein expression intensity of anti-oxidation protein was significantly decreased in CRS rats, and the treatment of budesonide and prednisolone decreased the mRNA expression and protein expression intensity of anti-oxidation protein. Also, there was no significantly difference between topical budesonide group and oral prednisolone treatment group.

Besides, TLR4/TRAF6/NF-κB signal pathway play an important role in the progress of CRS[32]. Furthermore, during inflammation, transcription factors are activated and translocate to the cell nucleus, inducing transcription of genes associated with inflammatory cytokines, chemokines, and adhesion molecules[33]. NF-κB is a vital pro-inflammatory nuclear transcription faction, whose active subunit p65 induces transcription of cytokines, chemokines, and adhesion molecules[34]. Signals from tumor necrosis factor receptor (TRAF) and toll-like receptor (TLR) superfamilies are integrated by the NF-κB signaling network to produce transcriptional responses[34]. In the present study, our results shown that with the upregulation of anti-oxidant proteins in the nasal mucosa of CRS rats, the expression of TLR4/TRAF6/NF-κB marker proteins was also upregulated. Also, treatment with budesonide and prednisolone decreased the protein expression of anti-oxidant and TRAF6/NF-κB marker, and there was no difference between topical treatment with budesonide and oral prednisolone.

Collectively, our results have confirmed the conclusion that the treatment effect of topical budesonide and oral prednisolone in CRS rats was no significant difference. For the therapeutic approach of CRS, patient can choice the medicine according to the potential side effect. While, more detailed studies are needed in the future to further prove our conclusions.

Funding

This work was supported by The Program Innovative Research Team in Science and Technology in Yunnan Province (No. 202005AE160004).

Reference

1. DeConde AS, Soler ZM. Chronic rhinosinusitis: Epidemiology and burden of disease. American journal of rhinology & allergy 2016, 30(2): 134-139.
2. Huang CC, Wang CH, Wu PW, He JR, Huang CC, Chang PH*, et al.* Increased nasal matrix metalloproteinase-1 and -9 expression in smokers with chronic rhinosinusitis and asthma. Scientific reports 2019, 9(1): 15357.
3. Cho SH, Ledford D, Lockey RF. Medical Management Strategies in Acute and Chronic Rhinosinusitis. The journal of allergy and clinical immunology In practice 2020, 8(5): 1559-1564.
4. Sedaghat AR. Chronic Rhinosinusitis. American family physician 2017, 96(8): 500-506.
5. Wallwork B, Coman W, Feron F, Mackay-Sim A, Cervin A. Clarithromycin and prednisolone inhibit cytokine production in chronic rhinosinusitis. The Laryngoscope

2002, 112(10): 1827-1830.

6. Poetker DM. Oral corticosteroids in the management of chronic rhinosinusitis with and without nasal polyps: Risks and benefits. American journal of rhinology & allergy 2015, 29(5): 339-342.
7. Ghogomu N, Kern R. Chronic rhinosinusitis: the rationale for current treatments. Expert review of clinical immunology 2017, 13(3): 259-270.
8. Smith KA, French G, Mechor B, Rudmik L. Safety of long-term high-volume sinonasal budesonide irrigations for chronic rhinosinusitis. International forum of allergy & rhinology 2016, 6(3): 228-232.
9. Evans DJ, Taylor DA, Zetterstrom O, Chung KF, O'Connor BJ, Barnes PJ. A comparison of low-dose inhaled budesonide plus theophylline and high-dose inhaled budesonide for moderate asthma. The New England journal of medicine 1997, 337(20): 1412-1418.
10. Leflein JG, Szefler SJ, Murphy KR, Fitzpatrick S, Cruz-Rivera M, Miller CJ*, et al.* Nebulized budesonide inhalation suspension compared with cromolyn sodium nebulizer solution for asthma in young children: results of a randomized outcomes trial. Pediatrics 2002, 109(5): 866-872.
11. Toogood JH, Baskerville JC, Jennings B, Lefcoe NM, Johansson SA. Influence of dosing frequency and schedule on the response of chronic asthmatics to the aerosol steroid, budesonide. The Journal of allergy and clinical immunology 1982, 70(4): 288-298.
12. Stanaland BE. Once-daily budesonide aqueous nasal spray for allergic rhinitis: a review. Clinical therapeutics 2004, 26(4): 473-492.
13. Daval M, Corré A, Palpacuer C, Housset J, Poillon G, Eliezer M*, et al.* Efficacy of local budesonide therapy in the management of persistent hyposmia in COVID-19 patients without signs of severity: A structured summary of a study protocol for a randomised controlled trial. Trials 2020, 21(1): 666.
14. Huang ZZ, Chen XZ, Huang JC, Wang ZY, Li X, Chen XH*, et al.* Budesonide nasal irrigation improved Lund-Kennedy endoscopic score of chronic rhinosinusitis patients after endoscopic sinus surgery. European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery 2019, 276(5): 1397-1403.
15. Jankowski R, Schrewelius C, Bonfils P, Saban Y, Gilain L, Prades JM*, et al.* Efficacy and tolerability of budesonide aqueous nasal spray treatment in patients with nasal polyps. Archives of otolaryngology--head & neck surgery 2001, 127(4): 447-452.
16. Juniper EF, Kline PA, Ramsdale EH, Hargreave FE. Comparison of the efficacy and side effects of aqueous steroid nasal spray (budesonide) and allergen-injection therapy (Pollinex-R) in the treatment of seasonal allergic rhinoconjunctivitis. The Journal of allergy and clinical immunology 1990, 85(3): 606-611.
17. Prednisolone. Journal of the American Pharmaceutical Association 1976, 16(3): 143-146.
18. Rodriguez-Martinez CE, Sossa-

Briceño MP, Castro-Rodriguez JA. Dexamethasone or prednisolone for asthma exacerbations in children: A cost-effectiveness analysis. Pediatric pulmonology 2020, 55(7): 1617-1623.

19. Poetker DM, Jakubowski LA, Lal D, Hwang PH, Wright ED, Smith TL. Oral corticosteroids in the management of adult chronic rhinosinusitis with and without nasal polyps: an evidence-based review with recommendations. International forum of allergy & rhinology 2013, 3(2): 104-120.
20. Barnes PJ. Inhaled corticosteroids in COPD: a controversy. Respiration; international review of thoracic diseases 2010, 80(2): 89-95.
21. Hafström I, Albertsson K, Boonen A, van der Heijde D, Landewé R, Svensson B. Remission achieved after 2 years treatment with low-dose prednisolone in addition to disease-modifying anti-rheumatic drugs in early rheumatoid arthritis is associated with reduced joint destruction still present after 4 years: an open 2-year continuation study. Annals of the rheumatic diseases 2009, 68(4): 508-513.
22. Hengge UR, Ruzicka T, Schwartz RA, Cork MJ. Adverse effects of topical glucocorticosteroids. Journal of the American Academy of Dermatology 2006, 54(1): 1-15; quiz 16-18.
23. Mitts KB, Chang EH. Genetics of chronic rhinosinusitis. The Journal of allergy and clinical immunology 2020, 145(3): 777-779.
24. Tait S, Kallogjeri D, Suko J, Kukuljan S, Schneider J, Piccirillo JF. Effect of Budesonide Added to Large-Volume, Low-pressure Saline Sinus Irrigation for Chronic Rhinosinusitis: A Randomized Clinical Trial. JAMA otolaryngology-- head & neck surgery 2018, 144(7): 605-612.
25. Sachanandani NS, Piccirillo JF, Kramper MA, Thawley SE, Vlahiotis A. The effect of nasally administered budesonide respules on adrenal cortex function in patients with chronic rhinosinusitis. Archives of otolaryngology--head & neck surgery 2009, 135(3): 303-307.
26. Hamilos DL. Drivers of chronic rhinosinusitis: Inflammation versus infection. The Journal of allergy and clinical immunology 2015, 136(6): 1454-1459.
27. Zheng K, Hao J, Xiao L, Wang M, Zhao Y, Fan D*, et al.* Expression of nicotinamide adenine dinucleotide phosphate oxidase in chronic rhinosinusitis with nasal polyps. International forum of allergy & rhinology 2020, 10(5): 646-655.
28. Lin H, Ba G, Tang R, Li M, Li Z, Li D*, et al.* Increased Expression of TXNIP Facilitates Oxidative Stress in Nasal Epithelial Cells of Patients With Chronic Rhinosinusitis With Nasal Polyps. American journal of rhinology & allergy 2020: 1945892420982411.
29. Hong G, Zheng D, Zhang L, Ni R, Wang G, Fan GC*, et al.* Administration of nicotinamide riboside prevents oxidative stress and organ injury in sepsis. Free radical biology & medicine 2018, 123: 125-137.
30. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V*, et al.* Oxidative Stress: Harms and Benefits for Human Health. Oxidative medicine and cellular longevity 2017, 2017: 8416763.
31. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals,

metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions 2006, 160(1): 1-40.

32. Zhou F, Liu P, Lv H, Gao Z, Chang W, Xu Y. miR-31 attenuates murine allergic rhinitis by suppressing interleukin-13-induced nasal epithelial inflammatory responses. Molecular medicine reports 2021, 23(1).

33. Lee JI, Burckart GJ. Nuclear factor kappa B: important transcription factor and therapeutic target. Journal of clinical pharmacology 1998, 38(11): 981-993.

34. Wang J, Cui Z, Liu L, Zhang S, Zhang Y, Zhang Y*, et al.* MiR-146a mimic attenuates murine allergic rhinitis by downregulating TLR4/TRAF6/NF-κB pathway. Immunotherapy 2019, 11(13): 1095-1105.