

Effects of glucocorticoid on the expression and regulation of aquaporin 5 in the paranasal sinus of rats with chronic rhinosinusitis

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Abstract. Aquaporins (AQPs) are water-specific membrane channel proteins that regulate water homeostasis for cells and organisms. AQP5 serves an important role in the maintenance of mucosal water homeostasis, and potentially contributes to mucosal edema and inflammation formation in chronic rhinosinusitis (CRS). The aim of the present study was to explore the expression pattern of AQP5 and the effect of glucocorticoids on AQP5 expression in rats with CRS. The rats were randomly divided into three equal groups, as follows: CRS, dexamethasone (dexa) treatment and control groups. A polyvinyl acetal material containing *Staphylococcus aureus* was inserted into the left nasal cavity of each rat from the CRS and dexa groups. On the 90th post-operative day, the dexa group received dexamethasone (2 mg/kg/day) via intraperitoneal injection for 7 days. The controls did not receive any treatment. The expression of AQP5 in the sinonasal mucosa was determined using immunohistochemistry and quantitative PCR. The immunoreactivities of AQP5 were primarily noted in the epithelial lining and glandular cells, the vascular endothelium and in the goblet cells in the sinonasal mucosa. The AQP5 mRNA expression level was significantly higher in the dexa group than in the control and CRS groups ($P=0.006$ and $P=0.014$, respectively). However, no significant difference was indicated between the CRS and control groups ($P=0.760$). In conclusion, the current study suggests that glucocorticoids induce AQP5 expression in

the sinonasal mucosa of CRS rats, which highlights AQP5 as a potential target in the diagnosis and treatment of CRS.

Introduction

Chronic rhinosinusitis (CRS) is a group of disorders defined as persistent inflammation involving the nose and paranasal mucosa, and is characterized by nasal blockage/obstruction/congestion, nasal discharge, facial pain/pressure, and/or reduction in or loss of smell (1). The overall prevalence of CRS ranged from 6.9 to 27.1% (mean, 10.9%) due to obvious geographical variation (2), which causes a significant public health problem as well as considerable socioeconomic costs (1,3). Currently, not all CRS cases can be completely controlled due to the complex and multifactorial etiology of this condition, including the involvement of exogenous pathogens, microbial biofilms and individual host factors (4,5).

Glucocorticoids are effectively used as anti-inflammatory drugs to improve airway inflammatory diseases, and are considered as the first-line treatment for CRS (1,6,7). In addition, glucocorticoids have been reported to regulate the water balance in various tissues and organs, including the lungs, peritoneum and middle ear (8).

Aquaporin 5 (AQP5) is a crucial protein formed by four subunits that passively transports water in and out of cells in accordance with the osmotic gradient across the membrane (9). AQP5 has been described to serve a role in several diseases associated with dysfunction of water regulation, and is key in this process (10-12). Furthermore, differences in the cellular location and mRNA expression pattern of AQP5 were observed in the nasal tissues from CRS patients compared with those of healthy controls (13,14).

Based on their hypothesized functions, glucocorticoids may also alleviate edema and improve the symptoms of CRS by means of their regulatory role on AQP5. No previous studies have examined the effects of glucocorticoids on AQP5 in CRS, to the best of our knowledge. In the present study, the expression pattern of AQP5 and the effect of glucocorticoids on AQP5 expression were studied using a rat model of CRS.

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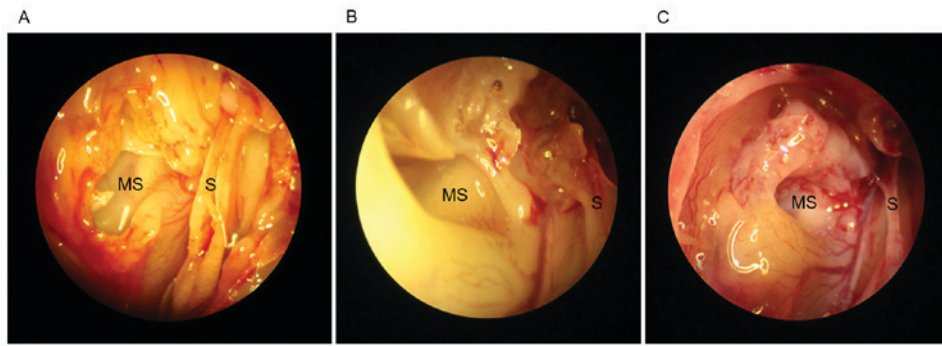


Figure 1. Images of nasoendoscopy in rats of (A) control; (B) CRS; and (C) dexamethasone groups. CRS, chronic rhinosinusitis; dexamethasone; MS, maxillary sinus; S, septum.

Materials and methods

Animal model and treatment. A total of 30 male Sprague-Dawley rats (age, 6 months; Animal Laboratory Center, Nanjing Drum Tower Hospital, Nanjing, China) weighing between 220 and 250 g were used in this study. All animals were handled according to the guidelines of the Animal Care and Use Committee of Nanjing Drum Tower Hospital, Nanjing University School of Medicine.

The rats were randomly divided into three equal groups, as follows: i) CRS; ii) dexamethasone (dexamethasone) treatment; and iii) control groups. Animals in the first two groups were anesthetized by intraperitoneal injection of a mixture of ketamine (50 mg/kg; Jiangsu Hengrui Pharmaceutical Co., Ltd., Jiangsu, China) and diazepam (5 mg/kg; Tianjin Jinyao Pharmaceutical Co., Ltd., Tianjin, China), and a polyvinyl acetal material (3x5 mm) with *Staphylococcus aureus* (ATCC 25923; American Type Culture Collection, Manassas, VA, USA), produced in the Laboratory of Clinical Microbiology, Nanjing Drum Tower Hospital) was inserted into the left nasal cavity of each rat from the CRS and dexamethasone groups. The control group did not receive any treatment. On the 90th postoperative day, the dexamethasone group received dexamethasone (2 mg/kg/day) via intraperitoneal injection for 7 days. All rats were sacrificed under anesthesia via an intraperitoneal injection of a mixture of ketamine (50 mg/kg) and diazepam (5 mg/kg) on the 97th postoperative day, and the left sinonasal mucosa were removed for subsequent experiments (Fig. 1). Some samples were used immediately for histology and immunohistochemical staining, others were stored in eppendorf tubes at -70°C .

Histology and immunohistochemical staining. The samples were prepared by cardiac perfusion with physiological saline, and were then fixed with 4% paraformaldehyde for 24 h at 4°C . The specimens were dehydrated by a graded ethanol series and embedded in paraffin. Tissues were cut into 4- μm sections, deparaffinized, and hydrated with phosphate-buffered saline (PBS; pH 7.4). A number of sections were stained with hematoxylin and eosin (HE) for morphological examination, as described previously (15), whereas other samples from the same rats were treated with 3% H_2O_2 for 30 min at room temperature to block the endogenous peroxidase, and then incubated with rabbit polyclonal antibody against AQP5 (ab92320; Abcam, Cambridge, UK; 1:800 dilution) for 18 h at 4°C . The sections were then washed with PBS and incubated

with a free biotin-conjugated anti-rabbit IgG secondary antibody (PV8000; PowerVision Two-Step Histostaining Reagent; Zhongshan Golden Bridge, Beijing, China; 1:100 dilution) for 30 min at room temperature. The sections were incubated with 0.05% 3,3'-diaminobenzidine and counterstained with Mayer's hematoxylin. The negative control was incubated with 0.01 M PBS instead of the primary antibody.

RNA extraction, reverse transcription, and quantitative PCR (qPCR). Total RNA of samples from the left sinonasal mucosa was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific Inc., Waltham, MA, USA), and 1 μg of total RNA was reverse-transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions. qPCR was conducted using specific primers (10 $\mu\text{mol/l}$; Invitrogen; Thermo Fisher Scientific, Inc.) and SYBR Premix Ex Taq kit (ABI, USA) with an ABI 7900HT Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The concentration of mRNA used in each reaction was 1 $\mu\text{g}/\mu\text{l}$. The sequences of the primers were as follows: AQP5 forward, 5'-AGGAGAGGAAGAAGACCATCGA-3', and reverse, 5'-TGCTTCAAACCTCTTCGTCTTCCTT-3'; β -actin forward, 5'-CCCATCTATGAGGGTTACGC-3' and reverse, 5'-TTT AATGTCACGCACGATTTTC-3'. The amplification reaction consisted of 40 cycles of denaturation (95° ; 20 sec), annealing (60° ; 60 sec) and extension (72° ; 60 sec). The level of mRNA was assessed using the comparative cycle threshold (Cq) method (16), relative to β -actin.

Statistical analysis. Statistical analyses were performed using SAS software 9.1.3 (SAS Institute Inc., Cary, NC, USA). Comparisons of relative mRNA expression between groups were analyzed using the unpaired Student's t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Histology. HE staining revealed no evidence of inflammatory clusters in the mucosa of the maxillary sinus obtained from control rats (Fig. 2A). However, erosions of epithelial cilia, gland damage, vessel dilation, edema and dispersed lymphocytes in the connective tissue were observed in the sinonasal mucosa in the CRS and dexamethasone groups (Fig. 2B and C), which confirmed chronic sinonasal inflammation.

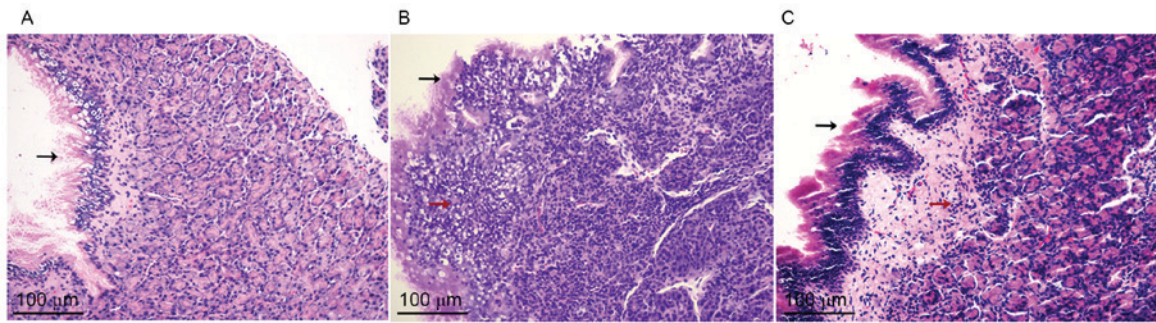


Figure 2. Hematoxylin and eosin staining of the rat sinonasal mucosa from the (A) control; (B) CRS; and (C) dexa groups. Black arrow, ciliated epithelium; red arrow, damaged glands and lymphocyte infiltration; x200 magnification. CRS, chronic rhinosinusitis; dexa, dexamethasone.

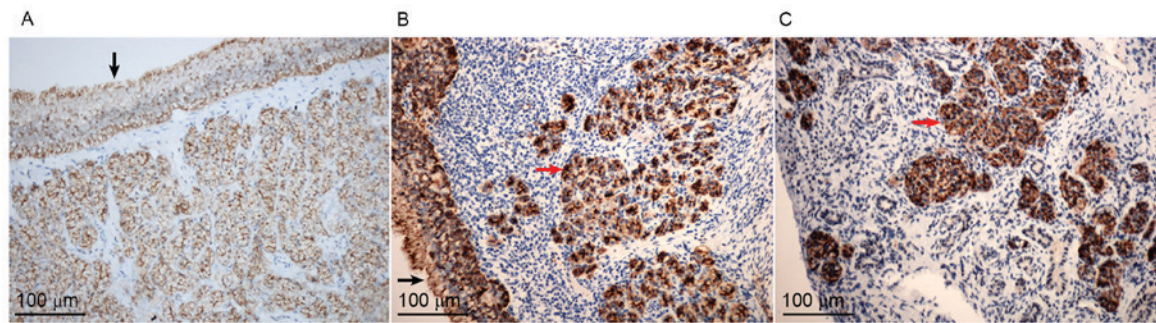


Figure 3. Immunohistochemical localization of aquaporin 5 in the sinonasal mucosa of rats from the (A) control; (B) CRS; and (C) dexa groups. Black arrow, ciliated epithelium; red arrow, glands; brown, positive staining; x200 magnification. CRS, chronic rhinosinusitis; dexa, dexamethasone.

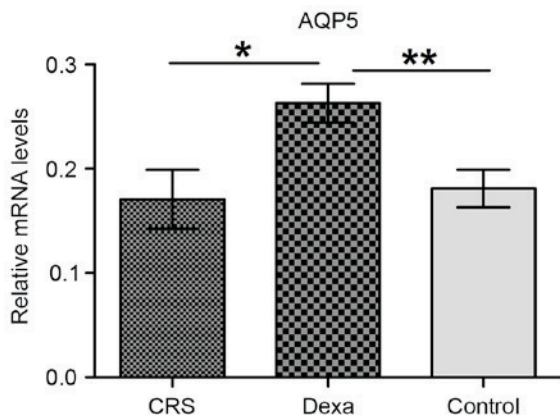


Figure 4. AQP5 mRNA expression levels in the sinonasal mucosa of rats from the CRS, dexa and control groups. Data are presented as the mean \pm standard error of the mean. * $P < 0.05$; ** $P < 0.01$. CRS, chronic rhinosinusitis; dexa, dexamethasone; AQP5, aquaporin 5.

Immunohistochemical staining. The sinonasal mucosal specimens from all three groups demonstrated positive AQP5 staining, as indicated by the brownish color (Fig. 3). The immunoreactivity of AQP5 was primarily noted in the epithelial lining, glandular cells, vascular endothelium and goblet cells in the sinonasal mucosa (Fig. 3A). No AQP5 expression was observed in the damaged gland areas demonstrating lymphocyte infiltration, whereas a marked reaction was observed in the residual glands (red arrows; Fig. 3B and C).

qPCR. AQP5 mRNA expression was confirmed by qPCR, and was detected in the sinonasal mucosa from all three groups. As

reported in Fig. 4, the AQP5 mRNA level was significantly higher in the dexa group than in the CRS and control groups ($P = 0.014$ and $P = 0.006$, respectively). However, no significant difference was found between the CRS and control groups ($P = 0.760$).

Discussion

Among the multiple etiological hypotheses of inflammation involving exogenous and host factors, bacterial colonization is the most cited factor (1,17,18). As the most common colonizer of the nasal passages and sinuses, *S. aureus* is also regarded as a primary causal pathogen of CRS, owing to its high prevalence in CRS patients (17,19), ability to secrete superantigens that alleviate airway inflammation (20) and tendency to form a biofilm, which negatively affects treatment outcomes in CRS patients (21).

In the present study, with the aim of stimulating and maintaining chronic sinonasal inflammation, a model of CRS was established in rats using intranasally administered *S. aureus*. The experimental CRS that developed in the current model is similar to that reported in previous studies (22,23).

AQP5 was confirmed to be primarily expressed in the epithelial lining, subepithelial glandular cells, vascular endothelium and goblet cells of the sinonasal mucosa of the rats. This distribution is consistent with previous findings in humans (14,24). The subepithelial glandular cells are established to participate in mucus secretion and consistency, which maintain the function of the mucociliary system. Furthermore, the epithelial cilia are crucial in osmotic transport to facilitate cilia-dependent movement of mucus. Thus, the present distribution pattern suggests that AQP5 serves notable and coordinated roles in osmotic homeostasis in the sinonasal mucosa.

AQP5 mRNA was also detected in the sinonasal mucosa of the rat model. Specifically, the AQP5 mRNA level in the dexta group was significantly higher than that of the other two groups, with no significant difference between the CRS and control groups. It is therefore hypothesized that the underlying reason for this result is as follows: The persistent inflammation induced by *S. aureus* damaged the natural structure of the nasal mucosa, including the ciliated epithelium and glandular tissue, and AQP5 was predominantly localized to these two cell types. It may be speculated that there may, in fact, be increased expression of AQP5 mRNA in the sinonasal mucosa of CRS rats; however, as the remaining cell counts were reduced in this model, there was an overall similar value of the mRNA quantity compared with the control group.

The valuable role of glucocorticoids in the conservative treatment of CRS is undeniable; in addition to their anti-inflammatory effects, glucocorticoids can regulate the water balance in multiple tissues (8,25,26). Water transport of the sinonasal mucosa is also noteworthy in the pathogenesis of CRS. Abnormal water homeostasis leads to nasal obstruction, purulent discharge and polyp formation. Previous studies revealed that glucocorticoids increased the expression of AQP5 in human airway epithelial cells (25), and may alleviate pulmonary edema in asthmatic rats (26).

In the present study, elevated expression of AQP5 mRNA was observed following glucocorticoid stimulation by intraperitoneal administration for 7 days. It was therefore hypothesized that the regulatory effect of glucocorticoids on AQP5 results in osmotic homeostasis of the nasal mucosa via promotion of glandular secretion and alleviating edema, which consequently improves local inflammation. However, additional studies are required to confirm the protein expression pattern of AQP5, and to elucidate whether glucocorticoids modulate the expression of AQP5 in a dose- and/or time-dependent manner.

In summary, the current study investigated the expression of AQP5 and the effect of glucocorticoids on AQP5 expression in a rat model of CRS. The results demonstrated that glucocorticoids enhance the functional expression of AQP5. These findings may provide evidence for a novel target in CRS treatment.

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