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The effect of topical intranasal H89 and dimethylsulfoxide on AQP5 levels and histopathological findings in an experimental allergic rhinitis rat model

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ABSTRACT

Aim: To investigate the protective effects of protein kinase A inhibitor H89 and its solvent, DMSO (dimethylsulfoxide), on the nasal mucosa of rats in an allergic rhinitis model.
Method: In total, 32 adult male Wistar albino rats were divided into four groups, with eight rats in each group. Ovalbumin (OVA) sensitization was used to induce allergic rhinitis. DMSO, a solvent of H89, was administered through a topical intranasal spray to the sham group. No treatment was involved in the allergic rhinitis group. H89 was administered through a topical intranasal spray to the H89 group. After the experiment, rat nasal tissues were stained with hematoxylin-eosin and AQP5 (Aquaporin 5) antibodies. Histopathological and immunohistochemical evaluations were performed under a light microscope.
Results: Vascular congestion, eosinophil infiltration, cilia loss, goblet cell proliferation, and degeneration in the mucosal glands were statistically significantly lower in the H89 group compared to the allergic rhinitis group. There was no statistical difference in the increase of connective tissue, vascular proliferation, or inflammatory cell infiltration. We posit that the histological improvements in the H89 group are due to the DMSO distribution of AQP5 was statistically significantly reduced in the H89 group compared to the allergic rhinitis group.
Conclusion: H89 reduces the level of AQP5 but does not lessen allergic manifestations in the mucosa. DMSO, which we used as a solvent, did not affect the AQP5 level but reduced nasal inflammation.

Key words: Aquaporin, H89, dimethylsulfoxide, ovalbumin, allergic rhinitis, protein kinase A.

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Introduction

Allergic rhinitis (AR) is a type 1 hypersensitivity reaction with chronic inflammation in the nasal mucosa due to IgE synthesis and mast cell degranulation. Symptoms ocur upon exposure to an allergen with immunological sensitivity [1,2]. The prevalence of allergic rhinitis has increased and affects approximately 40% of children and 10%-30% of adults. [1-4]. The incidence of sinusitis and otitis media increases in the presence of this chronic disease, which causes a decrease in the quality of life and job loss [1,4]. Since AR is a chronic disease with a high prevalence, its economic burden is heavy [3]. Exacerbation by environmental stimuli, tight junction disruption mediated by inflammatory cytokines, or antigen proteolytic activity are

causes of sinonasal epithelial barrier disruption [5].

AQP5 plays a crucial role in water transport on the apical surface of the alveolar epithelium, the upper respiratory tract, and the submucosal epithelium of the nasopharynx [4,6]. It is the primary water channel in the human nasal region and functions as a tight junction protein that maintains mucosal water homeostasis. AQP5 is closely associated with upper respiratory tract pathologies. It regulates fluid secretion during allergic inflammation [5,7,8]. AQP5 performs this function in the mucosa using the cAMP protein kinase A (PKA) pathway [9]. H89 is a potent, selective inhibitor of cAMP-dependent (PKA) and exerts its inhibitory effect by competitively binding to the kinase catalytic subunit [10].

DMSO is a widely used solvent for organic and inorganic agents. It prevents freeze-thaw injuries by penetrating the cell membrane. For this reason, it is frequently used for the cryopreservation of cells and tissues for laboratory and clinical applications [11]. Furthermore, DMSO has recently been found to have immunomodulatory and anti-inflammatory effects [12].

Inadequate and costly treatments fuel the search for alternative treatments for allergic rhinitis. This study aims to histopathologically investigate the effects of the topical intranasal application of H89 and its solvent, DMSO, on the nasal mucosa of rats in an experimental allergic rhinitis model.

Materials and methods

Animals and study design

SDU Medical Faculty Animal Experiments Local Ethics Committee approval was obtained for this study (Protocol No:06.09.2011/04). The rules regarding animal care and use specified in the Declaration of Helsinki were followed. Thirty-two male Wistar albino rats weighing 250–350 g were used in this study. During the experiment, animals were kept in a 12-hour light-dark cycle at 50% humidity and 22 ± 2◦C temperature conditions and fed a standard laboratory diet. Healthy animals without nasal secretions were included in the study. The animals were divided into four groups, with eight rats in each group. Group 1 (control) is the untreated group. An allergic rhinitis model was created for Group 2 (OVA+DMSO), and DMSO, the solvent of H89, was administered through a topical intranasal spray. For Group 3 (OVA), an allergic rhinitis model was created, and no medication was dispensed. In Group 4 (OVA+ H89), only H89 was administered through a topicalintranasal spray.

Experimental allergic rhinitis

Our model used ovalbumin (OVA) sensitisation to induce experimental allergic rhinitis in rats [13]. OVA (25µgr/ml) and 5mg/ml aluminium hydroxide as an adjuvant were prepared for intraperitoneal administration in 0.9% saline. Except for the control group, 1 ml of a 200 ml solution was administered intraperitoneally on the 1st, 2nd, 3rd, 7th, and 10th days. From the seventh day, 0.02 g of OVA was dissolved in 20 ml of saline, and a 10% solution was administered intranasally to both nostrils twice a day.

Intranasal administration of H89 and DMSO

DMSO solution (Amresco Inc., USA) was administered intranasally to the sham group as two puffs (0.1 ml per puff) in each nostril on the 8th, 9th, 10th, and 14th days after the first OVA application. One hour after each DMSO application, two puffs of the OVA solution were administered intranasally into each nostril. H89 (LC Laboratories, Woburn MA, USA) solution was administered intranasally to the H89 group

as two puffs in each nostril on the 8th, 9th, 10th, and 14th days after the first OVA administration. One hour after each intranasal administration of H89, two puffs of OVA solution were delivered intranasally into each nostril.

Histopathological and immunohistochemical analyses

At the end of the study, the rats were decapitated under 2% anaesthesia with 10% ketamine (Alfamin, Alfasan, Holland) administered intraperitoneally. Nasal mucosa tissue was obtained by sacrificing the rats and fixed in a 10% buffered formalin solution for histopathological and immunohistochemical examinations. Sections of 2–4 µm thickness were obtained from the prepared paraffin blocks using a sliding microtome (Leica SM2000R, Germany). Tissue samples were stained with haematoxylin-eosin and examined under light microscopy regarding cilia loss, vascular proliferation, inflammatory cell infiltration, eosinophil infiltration, connective tissue increase, mucosal gland degeneration, goblet cell increase, and vascular congestion parameters. Sections were obtained from paraffin-embedded tissues, placed on polylysine slides, and stained with AQP5 primary antibody (anti-AQP5 ab, BIOSS, USA). A semiquantitative evaluation assessed nasal concha tissue sections with a photomicroscope, and the observed receptor densities were determined.

Statistical analysis

The statistical analysis was performed using the SPSS 16.0 program. The groups were compared using a non-parametric test, the Kruskal–Wallis test, to analyse the histochemical findings obtained in the samples. The values were expressed as medians and min-max values. The Mann–Whitney U test was used to compare measurements between the two groups. The level of significance was defined as $p<0.05$.

Results

Histopathological analysis

The semiquantitative histopathological evaluation of the sections under the light microscope revealed no pathological condition in the control group except for normal histological findings (Figure 1). There was a significant decrease in cilia loss, goblet cell proliferation, eosinophil infiltration, vascular proliferation, inflammatory cell infiltration, and degeneration in the mucosal glands in the sham group, which received only DMSO, compared to the allergic rhinitis group (p=0.015, p=0.001, p=0.009, p=0.012, p=0.0007, p=0.004) (Figure 1). Cilia loss and goblet cell increase were observed in the allergic rhinitis group. Increases in eosinophils and inflammatory cells were observed in the lamina propria. Vascular congestion and edema were detected in the connective tissue. Compared to the control group, there was a statistically significant increase in edema, vascular congestion, and loss of cilia, goblet cells, and inflammation. (p=0.000, p=0.000, p=0.008, p=0.001, p=0.001, p=0.030, p=0.000) (Figure -1). There was a significant decrease in cilia loss, goblet cell proliferation, eosinophil infiltration, vascular congestion, and mucosal gland degeneration in the H89 treatment group compared to the allergic rhinitis group (p=0.015, p=0.003, p=0.013, p=0.049, p=0.026). However, no significant difference was observed between the sham group and the allergic rhinitis group regarding connective tissue increase and vascular proliferation (p=0.469, p=0.215) (Figure -1).

Immunohistochemical analysis

AQP5 staining was mild in the arterioles and veins located in the tissue sample of the control group. In the epithelial cells in the sham group, statistically moderate (++) AQP5 staining was observed. (++) AQP5 staining was also detected in the AR group. It was observed that AR and

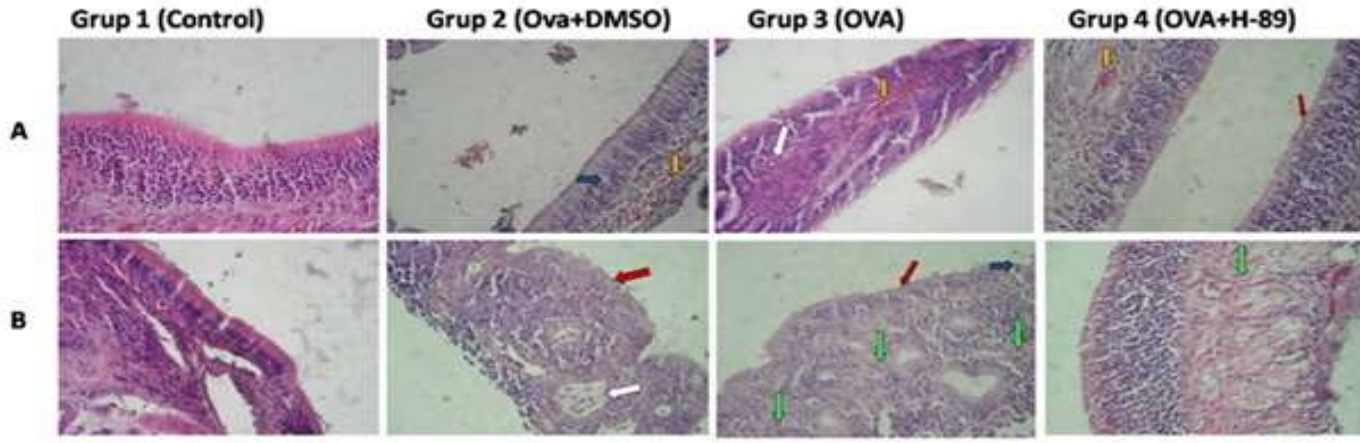

Figure 1. Effect of Ovalbumin (25µgr/ml), dimethyl sulfoxide (1ml) and H89 (100mg/kg/day) on nasal chonca. Histopathological sections from Group one (Control), Grup two (OVA+DMSO), Group three (OVA) and Group four (OVA+H89) showed near-normal nasal chonca histology. Histopathological sections from Control and Ova + DMSO group showed vascular congestion and proliferation (yellow arrow) and an increase in goblet cells (blue arrow) are seen in the vessels in the lamina propria layer. Loss of cilia (red arrow), congestion, proliferation and degeneration of the mucosal glands (White arrow) are seen in the epithelial cells of the nasal mucosa (H-E, X40). Histopathological sections from Control and OVA group showed cilia loss (red arrow) and goblet cell increase (blue arrow) in epithelial cells in the nasal mucosa, vascular congestion and proliferation (yellow arrow), eosinophilic and inflammatory cell infiltration (green arrow), increase and degeneration of mucosal glands (white arrow) in the connective tissue were detected (H-E, x20 and x40). Histopathological sections from OVA and Ova+H89 group showed an increase in the cilia of epithelial cells (red arrow) in the nasal mucosa, a decrease in goblet cell count, a decrease in vascular congestion and proliferation (yellow arrow), a decrease in the number of mucosal glands and degeneration, and a decrease in eosinophilic and inflammatory cell migration were detected (H-E, X40).

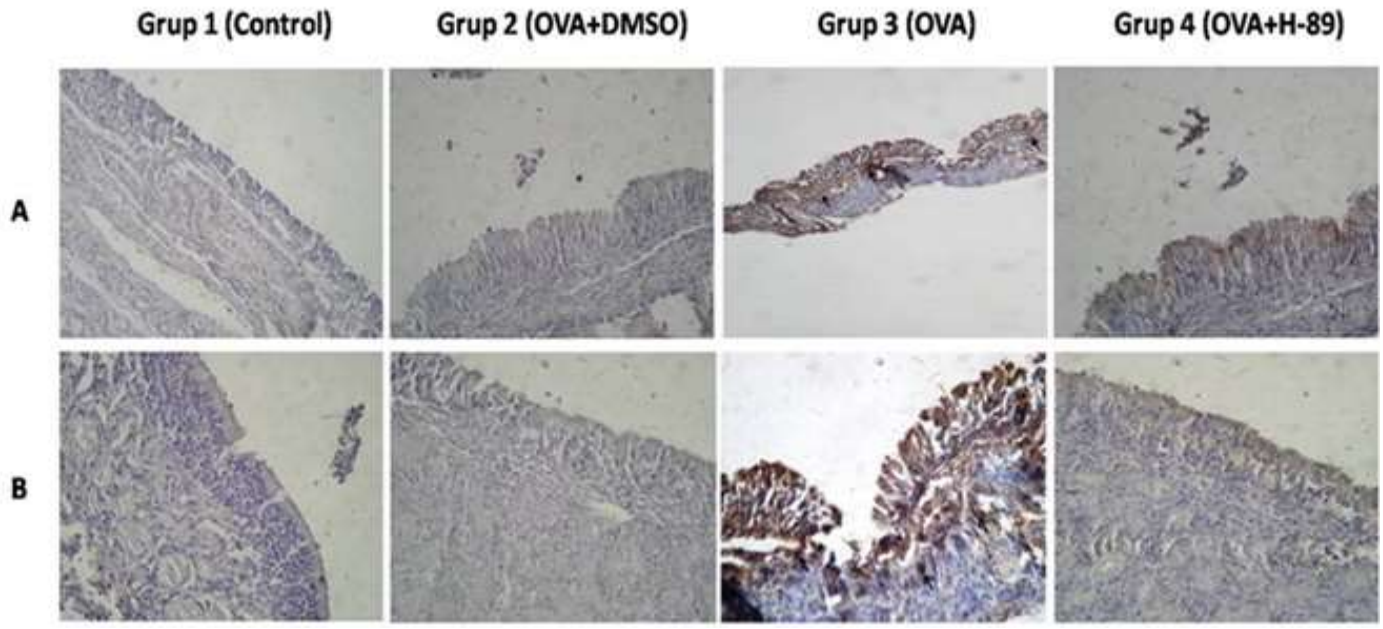

Figure 2. Effect of Ovalbumin (25µgr/ml), dimethyl sulfoxide (1ml) and H89 (100mg/kg/day) on nasal chonca. Immunohistological sections from Group 1 (Control), Group 2 (OVA+DMSO) and Group four (OVA+H89) showed less staining intensity. Conversely, sections from the Group three (OVA) showed intense staining intensity (aquaporin 5 immunostaining, black arrow). Control (A-X20 and B-X40), OVA+DMSO (A-X20 and B-X40), OVA (A-X20 and B-X40) and OVA+H89 (X40 and B-X40).

sham groups stained more in terms of AQP5 staining (p<0.05). In the H89 treatment group, mild (+) or no AQP5 staining was detected compared to the AR group. The H89 group was insignificant when compared to the control group (p>0.05), but it was statistically significant when compared to the sham group and AR group (p<0.05). The immuno-histochemical findings of all groups are presented in Figure 2.

Discussion

AR is an inflammatory disorder of the nasal mucosa caused by allergen exposure that triggers IgE-mediated inflammation. Mucosal inflammation causes venous dilatation, increased nasal secretion, and edema in the mucosa [14].

In the search for treatments, numerous animal experiments have been conducted to model allergic rhinitis. Various allergens have been used to cause allergic rhinitis in animal experiments. One of these substances is ovalbumin, called chicken egg albumin, and the other is Japanese cedar tree pollen [13]. In experimental allergic rhinitis models induced with ovalbumin, increased eosinophils in the nasal mucosa, vascular dilatation, congestion, edema and hyperplasia in the serous glands have been detected [15-18]. Immunohistochemical studies of the nasal tissues of patients with allergic rhinitis have revealed that dense eosinophils and mast cells accumulate in the lamina propria and epithelium [16]. Eosinophils are also present in the nasal secretions of patients with AR [19]. In our study, significant changes were found in the increase of goblet cells, vascular congestion, inflammatory cell infiltration, vascular proliferation, and eosinophil cell infiltration in three groups synthesized by giving ovalbumin, and allergic rhinitis was detected histologically (Figure 1).

Many studies confirm that AQPs are vital in maintaining fluid balance. AQP5 is mainly involved in maintaining water balance on the apical surface of the alveolar epithelium, the upper respiratory tract, and the submucosal epithelium of the nasopharynx [4,6,9]. AQP5 is closely associated with upper respiratory tract pathologies. It is the primary water channel in the human nasal region and plays a key role in fluid secretion during allergic inflammation [5,7,8]. Recent studies have determined that the cAMP-PKA/CREB pathway is involved in the regulation of AQP5 expression in the nasal epithelium of rats. It has been demonstrated that AQP5 performs this task in the mucosa by using the cAMP protein kinase A pathway, and it is possible to stop it at various stages [9]. Parvin et al. found that the vasoactive intestinal polypeptide significantly increased the amount of AQP5 in the duodenal apical membrane and that adding H89 blocked this increased expression [20]. Studies on mouse lung epithelial cells have revealed that H89 inhibits AQP5 synthesis [21,22]. A study performed on rat nasal epithelium observed that the number of AQP5 positive cells decreased after 12 or 24 hours of treatment with H89 [23]. Histamine, which induces hypersecretion in the nasal mucosa, plays the most important role in the pathophysiology of AR. In a study on human nasal epithelial cells, histamine reduced AQP5 expression [24]. Another study on human nasal epithelium found that histamine inhibited the expression of AQP5, while chlorpheniramine increased the levels of this protein dose-dependently [25]. Our study found that the H89 treatment group had mild or no AQP5 stainingcompared to the allergic rhinitis group. However, histopathological improvements were significant compared to the allergic rhinitis group. We posit that the histological

improvements in the H89 group are due to the DMSO we used as a solvent.

DMSO has been used not only as an industrial solvent but also as a medicine. Its various pharmacological functions have been demonstrated in laboratory studies. In clinical studies, it has alleviated several conditions, such as dermatological diseases, interstitial cystitis, and increased intracranial pressure. Recently, it has been found to have immunomodulatory and anti-inflammatory effects [12,26]. The anti-inflammatory activity of DMSO may be dose-dependent. In general, lower DMSO concentrations increase the sensitivity of the IFN-α receptor to its ligand, promote histamine release, and induce the infiltration of leukocytes [12,27,28].

In contrast, DMSO has been reported to suppress the inflammatory response by inhibiting the activation of NFκB and NLRP3 inflammasomes at high concentrations [29,30]. It has also been reported that DMSO suppresses the immune response by inducing the differentiation of regulatory T cells [12,31].

In our study, DMSO was more effective in correcting histopathological findings in allergic rhinitis due to its anti-inflammatory properties. Regarding vascular congestion, no improvement was observed in the group administered only DMSO.

Barrager et al. [32] found that the oral use of methylsulfonylmethane, the oxidative form of DMSO, is effective in allergic rhinitis. However, no study in the literature has focused on the topical use of DMSO in a rat model with allergic rhinitis. Since our study is a first in this respect, it cannot be compared with similar studies. The limitation of our study is that the effect and side-effect profiles of H89 and DMSO at different doses were not determined. In studies in which DMSO is used as a solvent, control and sham groups should be added, considering its anti-inflammatory activity.

Conclusion

We obtained significant results in the experimental treatment of allergic rhinitis. H89 reduces the level of AQP5 but does not lessen allergic manifestations in the mucosa. We found that DMSO, which we used as a solvent, did not affect the AQP5 level but reduced nasal inflammation. Therefore, the topical application of H89 and DMSO to the nasal mucosa of rats with allergic rhinitis expands the treatment options. However, further experimental and clinical studies are needed to determine the effects and side-effect profiles of H89 and DMSO.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *SDU Medical Faculty Animal Experiments Local Ethics Committee approval was obtained for this study (Protocol No:06.09.2011/04)*

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