The Efficacy of H89 on Aquaporin 5 levels in Asthmatic Rat Models

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Running head: Relation between Aquaporin 5 and H89 in Asthma

Synopsis: Asthma is a disease that progresses depending on the increased sensitivity in the respiratory tract and hypersecretion by goblet cells in bronchia. Inflammation sources that play a role in asthma's physiopathology are T lymphocytes, mast cells, eosinophils, and macrophages. Biopsy studies have revealed that there are correlations between density of inflammation and intensity of the diseases The effect of specific protein kinase A inhibitor H89 treatment reduced Aq 5 receptor expressions on the lung tissue.

ABSTRACT

Objective: The effect of specific protein kinase A inhibitor H89 on Aquaporin 5 levels, which has a role in the inflammation of asthma pathogenesis, was investigated in this study.

Material: 32 Wistar-Albino adult male rats, ranging between 250 and 350 grams, were divided into 4 groups: 1. Control group; 2. Shame group; administration of 1 ml Ovalbumin (OVA) solution intraperitonal (IP) and 0.1 ml OVA dissolved in dimethyl sulfoxide intranasal; 3. Asthma group; IP + intranasal OVA administration; and 4. H89 group; (IP + intranasal OVA) +0.1 ml H89. The lungs of the rats were evaluated histopathologically and immunohistochemically at the end of the study.

Results: The histopathological changes and AQ5 levels of the sham and asthma groups were not statistically different ($p > 0.05$). However, the parameters were found to be increased in the asthma group compared to the control group ($p < 0.001$). The alveolar degeneration and vascular congestion were statistically decreased in the H89 group ($p < 0.05$). The AQ5 levels were reduced in the H89 group, but the difference was not statistically significant.

Conclusion: Aquaporin 5 levels and histopathological changes were increased in asthmatic patients and an improvement was detected with H89 treatment. H89 has an effect on the inflammation of asthma pathogenesis, so it can be thought to be used in asthma treatment. However, more studies are needed to find out the therapy duration and ideal doses of H89 treatment.

Keywords: Asthma, Aquaporin 5, H89, Protein Kinase A, Ovalbumin.

INTRODUCTION

Asthma is a disease that progresses depending on the increased sensitivity in the respiratory tract and hypersecretion by goblet cells in bronchia. Inflammation sources that play a role in asthma's physiopathology are T lymphocytes, mast cells, eosinophils, and macrophages. Biopsy studies have revealed that there are correlations between density of inflammation and intensity of the diseases (1). The most prominent part is played by the corticosteroid in the suppression of inflammation in asthma treatment (2). These kinds of situations require new modalities to be applied in asthma treatment.

Protein Kinase A (PKA) causes specific genes to be overly expressed, and it also causes intracellular effects by phosphorylating specific proteins that bind to DNA binding sites (3). Aquaporin 5 (AQP5) is a member of the aquaporin family that is closely linked with secretion of the serous glands (4). It has been demonstrated that AQP5 levels are related with increased secretion, and AQP5 secretion is regulated with the cyclic adenosine monophosphate (cAMP)–PKA pathway (5, 6). In cell culture studies relevant to H89, a potent PKA inhibitor, important results on the PKA pathway have been attained, and these findings should be supported with in vivo studies.

We researched the H89 effect on AQP-5 levels that have a role in inflammation in asthma pathogenesis.

MATERIAL AND METHODS

Experimental group

This study was conducted in the Suleyman Demirel University Experimental Animals Laboratory. In total, 32 white albino Wistar rats that weighed between 250 gr and 350 gr were included in the study. Rats were separated into 4 groups;

Group 1 (Control): This is the group in which no action was taken.

Group 2 (Sham): Asthma was created by giving asthma with chicken serum albumin (OVA, grade III) and DMSO to this group.

Group 3 (Asthma): Experimental asthma model was constructed after the rats were sensitized with chicken serum albumin (OVA, grade III), and no other medicine was given to this group.

Group 4 (H89): This is the group that received only the medicine called H89 (LC laboratories Woburn MA, USA) in the form topical intranasal spray after being sensitized with chicken serum albumin.

Ketamine (Alfamin, Alfasan, Holland) IP at a dose of 90 mg/kg and xylazin IP at a dose of 10 mg/kg 24 hours after the last use of H89 anesthesia were applied to all of the rats. Following the anesthesia, lung tissues were placed in formaldehyde and examined histopathologically.

Preparation of Medicines

For the Sham group, a 0.9% physiological saline solution of 200 ml that contained OVA (25µgr/ml) and aluminumhydroxide (5mg/ml) was prepared. On days 1, 2, 3, 7, and 10, 1 ml (IP) of solution was given. Prepared DMSO solution 8, 9, 10, and 14 days after the first use of OVA (IP) was applied to each nostril in 2 doses. Thus, 2 doses of 0.1 ml DMSO solution were applied

in each nostril. Then, 1 hour after each DMSO application, OVA solution in which 0.02 gr OVA was solved in 20 ml SF was again given to each nostril in 2 doses.

The OVA sensitization method was employed, and the OVA solution was given to group 2, group 3, and group 4 on days 1, 2, 3, 7, and 10 in 1 ml (IP) in order to generate an experimental asthma model in rats. From day 7 on for inhalation use, the OVA solution was given to each rat's nostrils on days 8, 9, 10, and 14 in 2 doses of 0.1 ml each twice a day in group 2, group 3, and group 4.

The OVA solution was given to group H89 on days 1, 2, 3, 7, and 10 in 1 ml (IP). Prepared H89 (Sigma Aldrich) (15 ml solution of 30 micro molar) solution 8, 9, 10, and 14 days after the first use of OVA (IP) was applied to each of the nostrils in 2 doses. Then, 1 hour after each H89 use, OVA solution was applied given to each nostrils in 2 doses. The lungs of the rats were examined histopathologically and immunohistochemically after the experiment.

All procedures in this study was conducted in accordance with the National Institutes of Health laws and approved by the Suleyman Demirel University Animal Research Ethical Committee.

Histopathological and Immunohistochemical Analysis

Tissues were fixed with formaldehyde for histological parameters and examined under normal tissue microscope by staining with hemotoxylin-eosine. Stained samples were examined under an Olympus BX50 binocular microscope and evaluated by taking microphotographs from fractions. Fractions of each rat were scored separately from the point of the alveole degeneration, mononuclear cell infiltration, and vascular congestion. Lung tissues were fixed with 4%

paraformaldehyde solution for immunohistochemical analyses, and their Aquaporin 5 levels were observed.

Histopathological and imunohistochemical scoring was performed semiquantatively as in the study by Ercan et al. (7) and presented as below;

(0) score (negative score), no structural damage; (1) score (one positive score), minimal damage; (2) score (two positive scores), middle damage; (3) score (three positive scores): severe damage

Statistical Analysis

Statistical analyses were carried out by taking the benefit of SPSS 15.0 for Windows program. Histopathological and immunohistochemical results were evaluated statistically with Mann-WhitneyU test, correlations were evaluated with parametric t-test. p value less than 0.05 was considered significant. The $p < 0.05$ value was accepted as meaningful for the different results.

RESULTS

Histopathological and imunohistochemical scoring was shown in Table 1. Histopathological findings were not confronted in lung tissue samples of rats in the control group (Figure 1A). In the sham group, histopathological changes, such as alveolar degeneration, vascular congestion, hemorrhage, and mononuclear cell infiltration (MCI), were observed ($p<0.05$) (Figure 1B). A meaningful increase was determined in histopathological findings acquired in the asthma group called ovalbumin ($p<0.05$) (Figure 1C). A meaningful decrease existed in alveolar degeneration and vascular congestion in the treatment group called H89 ($p<0.05$) (Figure 1D).

In immunohistochemical analysis of tissue sections, it has been observed that AQP5 staining is slightly present alveolar of lung tissue in the control group and is the light (+) in the sham group (Figure 2A and Figure 2B). A non-statistically significant increase with AQP5 staining was detected in the alveolar cells in the asthmatic group ($p > 0.05$) (Figure 2C). Although it has been observed as decreasing to the levels of AQP5 staining in the alveolar cells in the treatment group to whom we gave H89 compared to the asthma group ($p > 0.05$) (Figure 2D).

DISCUSSION

Because asthma is a chronic inflammatory disease, existing drugs must be used effectively and over the long term (8). The significant side effects have risen due to the chronic usage of these drugs, which has led us to develop new therapeutic drugs. In the treatment of allergic and inflammatory diseases, the prolonged usage of glucocorticoids has limited the use of drugs because they cause major side effects, such as suppression of the immune system, peptic ulcer, hypertension, and osteoporosis. In addition, these drugs have ultimately created non-response to treatment due to glucocorticoid resistance evolving over time. This is the evidence of the need to develop new drugs, which have fewer side effects and are as effective as steroids (9-11). Due to all of this information, the main objective of this study is to prove that H89, which was thought to be a promising agent, may show anti-inflammatory activity in the treatment of asthma by causing inhibition of the protein kinase A enzyme that is involved in inflammation.

In the asthma study induced with ovalbumin conducted with this inflammation that occurrs in asthma carried out by Rogerio and colleagues in 2006, mononuclear cell infiltration

has been observed in lung histopathology. In addition, the substances called quercetin and isoquercitrin have been found to significantly suppress this inflammation (12).

In the study carried out by McKay and her colleagues, it was found that the perivascular and peribronchial cell infiltration was suppressed by simvastatin in an ovalbumin-induced asthma model (13). In another ovalbumin-induced study, it has been discussed that the protein kinase enzyme inhibition had reduced the T helper 2 cytokine production in asthma, the pulmonary eosinophilia, serum IgE and IgG1 synthesis, and mucus hypersecretion, and it also led to increased sensitivity of the respiratory tract (14). It was shown that the phosphodiesterase type 4 inhibitor (PDE4) rolipram and the adenylate cyclase activator forskolin, protein kinase A inhibitor H89, had suppressed the cAMP-induced eosinophilic infiltration (15). Hsieh and his colleagues damaged the liver with estradiol and bovine serum albumin, and they found that the protein kinase inhibitor H89 reduced the damage and hemorrhage (16). In a study in which the steroid was used together with the H89, it was found that the airway inflammation and hyperresponsiveness reduced. It was supposed that this dual therapy might cause a reduction in the therapy dose and the duration of glucocorticoid so that the steroid-related side effects might be reduced. It had been pointed out that H89 could be used in steroid resistance asthma treatment, depending on the results of this study (17).

In this study, in the ovalbumin-induced asthma model, a statistically significant reduction was present in the histopathological findings, such as alveolar degeneration, vascular congestion, hemorrhage, and mononuclear cell infiltration, which occurred in the lung tissue with the H89 treatment.

As associated with inflammation developed on the basis of the disease, an increase in the level of AQP5 was formed (4-6, 18). The water channel protein AQP5, which was firstly detected

in the submandibular gland (19), is also found in many tissues, such as apical membranes of the submucosal gland secretory cells, corneal epithelium, type I alveoli, and the lacrimal and salivary glands (20). The dysfunction of AQP5 has been associated with many diseases, such as asthma, hyperhidrosis, hipohidrosis, salivary disorders, and Sjögren's syndrome (21-23). In human beings, AQP5 is important, especially in the lungs and respiratory tract diseases. AQP5 has been localized in rat lungs in the epithelial cells' apical membranes lining type 1 alveolar (24). According to recent studies, the abundance and distribution of AQP-5 are regulated through the cAMP-PKA pathway (21, 25). The implementation of an anti-inflammatory property drug by inhibiting this cAMP-PKA pathway can lead to decreased levels of AQP5. For example, Yang and colleagues, in their cell culture study, found that AQP5 mRNA's protein levels and translocation of AQP5 to the apical plasma membrane had increased four-fold in cells inserted with chlorophenylthio-cAMP (cpt-cAMP), and they also showed that a specific protein kinase A inhibitor (H89) added to the cell culture decreased this effect (26). Sidhaye and his colleagues' cell culture study, which included lung epithelial cells, showed that terbutaline and forskolin, which raised cAMP levels, increased the amount of AQP5 and H89, which reduced the increased AQP5 levels (27). Parvin and colleagues also showed that AQP5 increased significantly and H89 blocked the increased expression when H89 was inserted in vitro into the cell culture of duodenal sections of an apical cell membrane that was treated with vasoactive intestinal polypeptide (VIP) (28). It was observed that AQP5 inhibited the feature of H89 in all of the mentioned studies, which were compatible with our study. In the in vivo study of the ovalbumin-induced lung inflammation model, it was revealed that the increased AQP5 levels in type I alveolar cells decreased as a result of the H89 treatment.

CONCLUSION

In light of all these findings, the reduction of the AQP5 expression, which is one of the indicators of inflammation, may be important in the anti-inflammatory activity and may contribute to the development of new drugs. Thus, we think that in the experimental ovalbumin-induced asthma model, protein kinase A enzyme inhibitor H89 suppresses inflammation; thus, it may be an alternative agent in the treatment of asthma. It is obvious that this suppression should be supported by the studies with different doses and durations in the future.

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Table 1: Histopathological and immunohistochemical analysis of lungs

	Control (n=8)				Sham (OVA+DMSO) (n=8)				Asthma (OVA) (n=8)				H89 (OVA+H89) (n=8)			
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Alveolar degeneration	8	0	0	0	0	1[a]	5[a]	2	0	0	6[b]	2[b]	0	5[c]	3[c]	0
Mononuclear cell infiltration	8	0	0	0	0	1[a]	6[a]	1[a]	0	2[b]	5[b]	1[b]	0	5	3	0
Vascular Congestion	4	4	0	0	0	3[a]	5[a]	0	0	1[b]	6[b]	1[b]	0	6[c]	2[c]	0
Hemorrhage	4	4	0	0	0	1[a]	6[a]	1[a]	0	2[b]	5[b]	1[b]	0	5	3	0
AQP5 staining	6	2	0	0	2[a]	5[a]	1[a]	0	0	6[b]	2[b]	0	2	5	1	0

a: Sham group induced lung injury, $p < 0.05$ vs. control.
b: Asthma induced lung injury, $p < 0.05$ vs. control.
c: Protective effect of H89, $p < 0.05$ vs.asthma.

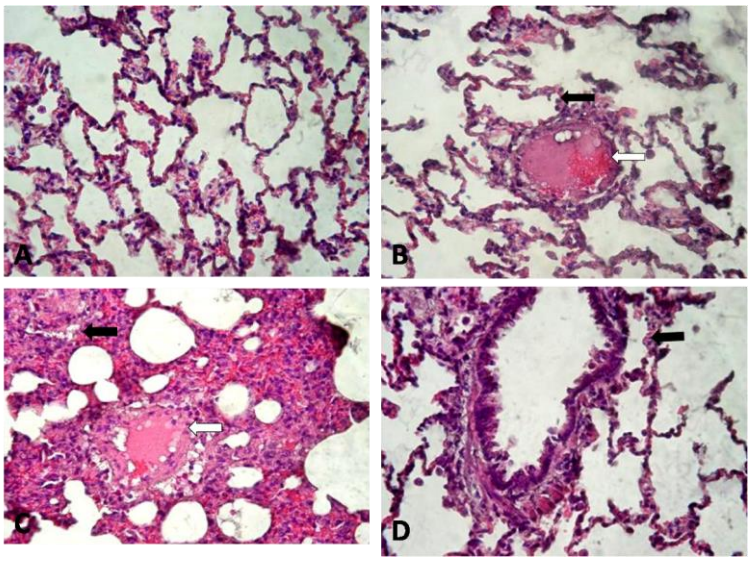

Fig. 1A: Image of lung tissue in control group (H-E; 40X). Figure 1B: Image of lung tissue in sham group. Hemorrhage (white arrow), alveolar degeneration (black arrow) (H-E; 40X).Figure 1C: Image of lung tissue in asthmatic group. Hemorrhage (white arrow), alveolar degeneration (black arrow) (H-E; 40X). Figure 1D: Image of lung tissue in asthmatic + H89 group. Alveolar degeneration (black arrow).

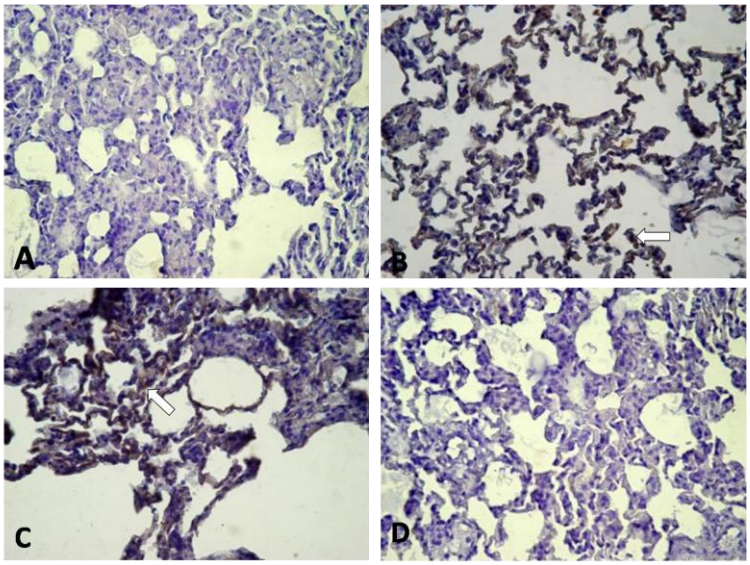

Figure 2A: Image of lung tissue in control group. AQP-5 staining negative immunohistochemical staining; 40X). **Figure 2B:** Image of lung tissue in sham group. AQP-5 staining positive (appearing as brown in the cell membranes of the alveoli (white arrows), (immunohistochemical staining; 40X). **Figure 2C:** Image of lung tissue in asthmatic group. AQP-5 staining positive (appearing as brown in the cell membranes of the alveoli (white arrows) (immunohistochemical staining; 40X). **Figure 2D:** Image of lung tissue in asthmatic + H89 group. Decreasing in AQP-5.