ORIGINAL ARTICLE

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

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ABSTRACT

Background: *The effect of a specific protein kinase A inhibitor H89 on Aquaporin 5 (AQ5) levels, which has a role in the inflammation of asthma pathogenesis, was investigated in this study.*

Objective: *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.*

Methods: *Thirty-two Wistar-Albino adult male rats, ranging between 250 and 350 g, were divided into four groups: (a) control group; (b) sham group, administration of 1 ml ovalbumin (OVA) solution intraperitonal (IP) and 0.1 ml OVA dissolved in dimethyl sulfoxide intranasally; (c) asthma group, IP + intranasally OVA administration; and (d) H89 group, (IP + intranasally 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 with the control group (*$p < 0.001$*). The alveolar degeneration and vascular congestion were statistically significantly 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, ovalbumin, protein kinase A

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 the 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 deoxyribonucleic acid-binding sites (3). Aquaporin 5 (AQP-5) is a member of the aquaporin family that is closely linked with the secretion of the serous glands (4). It has been demonstrated that AQP-5 levels are related with increased secretion, and AQP-5 secretion

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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.

MATERIALS 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 and 350 g were included in the study. Rats were separated into four 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 (ovalbumin [OVA], grade III) and dimethyl sulfoxide (DMSO) to this group.
Group 3 (Asthma): an 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 of topical intranasal spray after being sensitized with chicken serum albumin.

Ketamine (Alfamin, Alfasan, Holland) intraperitoneal (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 anaesthesia were applied to all of the rats. Following the anaesthesia, 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 (25 μg/ml) and aluminium hydroxide (5 mg/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 two doses. Thus, two doses of 0.1-ml DMSO solution were applied in each nostril. Then, 1 hour after each DMSO application, the OVA solution, in which 0.02-g OVA was solved in 20-ml sulfide, was again given to each nostril in two doses.

The OVA sensitization method was employed, and the OVA solution was given to groups 2–4 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 two doses of 0.1 ml each twice a day in groups 2–4.

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

All procedures in this study were 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 a normal tissue microscope by staining with hemotoxylin and 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 alveoli degeneration, mononuclear cell infiltration (MCI) and vascular congestion. Lung tissues were fixed with 4% paraformaldehyde solution for immunohistochemical analyses, and their AQP-5 levels were observed.

Histopathological and immunohistochemical scoring was performed semi-quantitatively as in the study by Ercan *et al* (7) and presented as follows:
(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 Statistical Package for Social Sciences version 15.0 (IBM, Chicago, IL, USA) for Windows program. Histopathological and immunohistochemical results were evaluated statistically with the Mann–Whitney U-test, and correlations were evaluated with the

parametric *t*-test. The *p*-value less than 0.05 was considered significant and was accepted as meaningful for the different results.

RESULTS

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

In immunohistochemical analysis of tissue sections, it has been observed that AQP-5 staining is slightly present in the alveolar of lung tissue in the control group and is light (+) in the sham group (Fig. 2A and 2B). A non-statistically significant increase with AQP-5 staining was detected in the alveolar cells in the asthmatic group ($p > 0.05$) (Fig. 2C). However, it has been observed as decreasing to the levels of AQP-5 staining in the alveolar cells in the treatment group to whom we gave H89 compared with the asthma group ($p > 0.05$) (Fig. 2D).

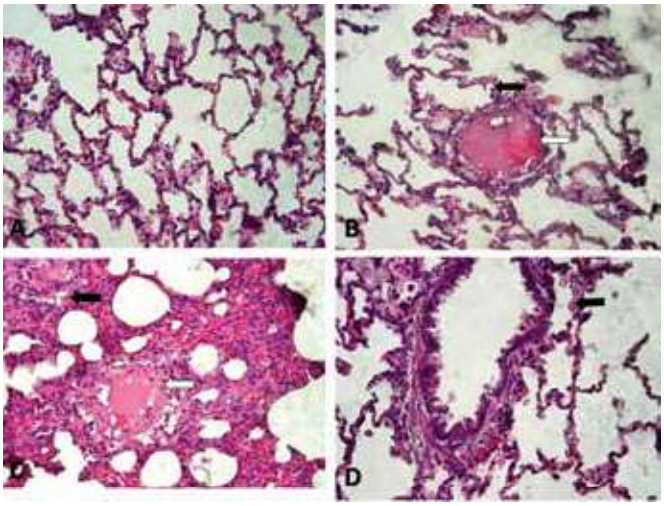

Figure 1. (A) Image of lung tissue in the control group (H&E; 40×). (B) Image of lung tissue in the sham group: haemorrhage (white arrow) and alveolar degeneration (black arrow) (H&E; 40×). (C) Image of lung tissue in the asthmatic group: haemorrhage (white arrow) and alveolar degeneration (black arrow) (H&E; 40×). (D) Image of lung tissue in the asthmatic + H89 group: alveolar degeneration (black arrow).
H&E = hemotoxylin and eosine.

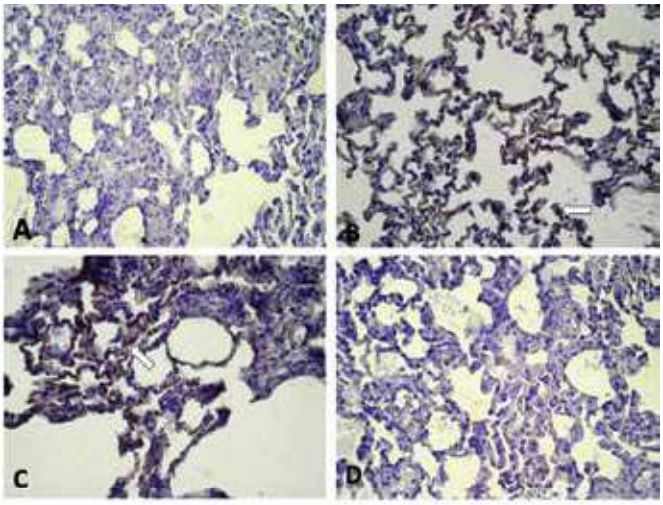

Figure 2. (A) Image of lung tissue in the control group: AQP-5 staining negative immunohistochemical staining; 40×). (B) Image of lung tissue in the sham group: AQP-5 staining positive (appearing as brown in the cell membranes of the alveoli (white arrows), (immunohistochemical staining; 40×). (C) Image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

Table: 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
Haemorrhage	4	4	0	0	0	1 a	6 a	1 a	0	2 b	5 b	1 b	0	5	3	0
AQP-5 staining	6	2	0	0	2[a]	5[a]	1[a]	0	0	6[b]	2[b]	0	2	5	1	0

AQP-5 = aquaporin 5; DMSO = dimethyl sulfoxide; OVA = ovalbumin.

[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.

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 reason 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 PKA enzyme that is involved in inflammation.

In the asthma study induced with OVA and conducted with this inflammation that occurs in asthma carried out by Rogerio *et al* in 2006, MCI was observed in lung histopathology. In addition, the substances called quercetin and isoquercitrin were found to significantly suppress this inflammation (12).

In the study carried out by McKay *et al* (13), it was found that the perivascular and peribronchial cell infiltration was suppressed by simvastatin in an OVA-induced asthma model. In another OVA-induced study, it was 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 the increased sensitivity of the respiratory tract (14). It was shown that the phosphodiesterase type 4 inhibitor, rolipram, and the adenylate cyclase activator forskolin, PKA inhibitor H89, had suppressed the cAMP-induced eosinophilic infiltration (15). Hsieh *et al* (16) damaged the liver with oestradiol and bovine serum albumin and found that the protein kinase inhibitor H89 reduced the damage and haemorrhage. In a study in which the steroid was used together with the H89, it was found that the airway inflammation and hyper-responsiveness 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 OVA-induced asthma model, a statistically significant reduction was present in the histopathological findings, such as alveolar degeneration, vascular congestion, haemorrhage and MCI, 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 AQP-5 was formed (4–6, 18). The water channel protein AQP-5, which was first detected in the submandibular gland (19), was 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 AQP-5 has been associated with many diseases, such as asthma, hyperhidrosis, hipohidrosis, salivary disorders and Sjögren's syndrome (21–23). In human beings, AQP-5 is important, especially in the cases of lungs and respiratory tract diseases. Aquaporin 5 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 AQP-5. For example, Yang *et al* (25), in their cell culture study, found that AQP-5 messenger ribonucleic acid's protein levels and translocation of AQP-5 to the apical plasma membrane had increased fourfold in cells inserted with chlorophenylthio-cAMP (cpt-cAMP), and they also showed that a specific PKA inhibitor (H89) added to the cell culture decreased this effect. Sidhaye *et al*'s cell culture study (26), which included lung epithelial cells, showed that terbutaline and forskolin, which raised cAMP levels, increased the amount of AQP-5 and H89, which reduced the increased AQP-5 levels. Parvin *et al* (27) also showed that AQP-5 significantly increased 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. It was observed that AQP-5 inhibited the feature of H89 in all of the mentioned studies, which were compatible with our study. In the in vivo study of the OVA-induced lung inflammation model, it was revealed that the increased AQP-5 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 AQP-5 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 an experimental OVA-induced asthma model, the PKA 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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