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# Changes in water channel aquaporin 1 and aquaporin 5 in the small airways and the alveoli in a rat asthma model

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ABSTRACT

*Objectives:* To examine changes in aquaporin 1 (AQP1) and aquaporin 5 (AQP5) in the small airways and alveoli in a rat asthma model.
*Method:* Forty Wistar rats were randomly divided into a control group and an ovalbumin (OVA) sensitization asthma model group. The distribution and expression of AQP1 and AQP5 in lung tissues were analyzed using immunohistochemistry (IHC), quantified the staining intensity by assessing integrated optical densities (IOD), and Western blotting (WB).
*Results:* IHC showed AQP1 was mainly distributed in sub-epithelial microvascular endothelial cells (MECs) and red blood cells. IOD values showed, in the asthma model group, the expression of AQP1 in alveolar MECs was lower than that in the control group ($P < 0.05$); However, AQP1 expression in small airways sub-epithelial was higher than in the control group ($P < 0.05$). The WB indicated that AQP1 expression in the asthma model group was 57% lower than in the control group ($P < 0.05$). AQP5 was mainly distributed in the non-ciliated epithelial cells of the small airways and the apical membranes of type I and type II epithelial cells. IOD values showed, in asthma model group, the expression of AQP5 increased in small airways epithelium ($P < 0.05$), and decreased in alveolar epithelium ($P < 0.05$). The WB showed a 36% reduction in AQP5 expression compared with the control group ($P < 0.05$).
*Conclusion:* AQP1 and AQP5 increased in small airways in rats with experimentally induced asthma, indicating that they may be involved in the formation of submucosal edema and mucus hypersecretion. Decreased AQP1 and AQP5 in pulmonary alveoli may be related to increased alveolar liquid viscosity and the formation of mucus plugs.



## 1. Introduction

Bronchial asthma (Asthma) remains one of the most common chronic respiratory diseases and it is associated with increasing morbidity and mortality in population. The main symptoms of asthma include airway inflammation, airway hyperactivity, mucus hypersecretion, mucous edema and airway remodeling (Sullivan et al., 2007). It has been documented that, the distal lung, including small airways (small airways being defined as airways <2 mm in internal diameter without cartilage), is actively contributes to enhanced airway hyperresponsiveness. Aquaporins (AQPs) are membrane water channels and it plays an important role in water transfer in airway system. Water transport across pulmonary epithelia is a vital component of lung physiology. Disorders of water metabolism are thought to result in mucus hypersecretion and mucous edema, although the exact mechanism has not been fully elucidated (Fahy and Dickey, 2010).

To date, 13 different AQPs have been identified in mammalian cells. In airway system, AQP1 is localized in microvascular endothelial cells (MECs) and the surrounding connective tissue cells, AQP5 in the apical side of epithelium cells (Matsuzaki et al., 2009; Ablimit et al., 2006). In the pulmonary, AQP1 and AQP5 provide the principal route for osmotically driven water transport. AQP1 may participate in the regulation of the permeability of plasma to the vascular walls (Bai et al., 1999), while AQP5 may be involved in the formation of mucus in the respiratory tract (King and Agre, 1996; Nielsen et al., 1997; Matsuzaki et al., 2009). However, their involvement in asthma remains to be clarified. In this study the expression of AQP1 and AQP5 was investigated in the lung tissue of rats with experimentally induced asthma.

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## 2. Material and methods

### 2.1. Animal model and tissue preparation

Male Wistar rats about 4 weeks old weighing about 200 g were purchased from the experimental animal center of Xinjiang Medical University. Rats were randomly divided into two groups of 15 each. One group was treated according to the methods of Vanacker et al. (2001). These rats were sensitized by intraperitoneal injection of 100 mg ovalbumin (OVA; Grade II Sigma–Aldrich) and 100 mg aluminum hydroxide ($Al(OH)_3$, Jinshan Chemical Plant, Shanghai, China) in 1 mL saline on Days 0 and 7. On Day 15, the rats were challenged with inhaled nebulized 1% OVA for 30 min. This was repeated every other day for 30 days. Animals in the control group received 0.9% saline.

Animals were anesthetized by intraperitoneal injection of 70 mg/kg sodium pentobarbital in saline within 24 h of the last treatment. Specimens were prepared from the right upper lobe of the lung and stored at −80 °C for protein measurement. The rest of the lung samples was cut into blocks and immersed in 4% formaldehyde solution for fixation. They were washed with sucrose solution for 12 h and then dehydrated and embedded in paraffin for H&E staining and immunohistochemistry. Paraffin-wax sections (4 μm thick) were cut, dewaxed and stained with H&E for pathological observation and cell counting. Five regions were randomly selected from each H&E section and observed for the presence of bronchioles 0.2 mm in diameter. Counts of imflammatory cells, lymphocyte and eosinophilic granulocyte were performed.

### 2.2. Immunohistochemistry

Primary antibodies used were as follows: AQP5 RabMab (abcam, ab78466), AQP1 RabMab (abcam, ab15080) and anti-surfactant protein A antibodies (SP-A). Secondary antibodies used were as follows: Rhodamine Red X-labeled donkey anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA); fluorescein isothiocyanate-labeled donkey anti-mouse IgG (Jackson Immunoresearch) and IgG (H + L)-HRP (horseradish peroxidase)-labeled goat anti-rabbit (Dako, P0488). For immunoperoxidase labeling, antigen retrieval with citrate was undertaken followed by 3% hydrogen peroxide treatment to eliminate endogenous peroxidase activity. Sections were first blocked with 1% bovine serum albumin (BSA) for 20 min, and then incubated with the primary antibody for 2 h. After washing with PBS (five-times in 5 min) the sections were incubated with the peroxidase-labeled secondary antibody for 1 h and washed with PBS. DAB solution (Dako, K3467) was then added for coloration. For immunofluorescence labeling, the non-specific binding of antibodies was also blocked by 1% BSA. The slides were then sequentially incubated with primary antibodies diluted in PBS containing 1% BSA overnight at 4 °C and fluorescently labeled secondary antibodies for 90 min at room temperature.

Image acquisition and analysis: after immunoperoxidase labeling, AQPs expression intensity was assessed by estimating the area of the objects and the medium pixel intensity per object, as the integrated optical density (IOD). The sections were imaged with a Leica DM 6000B microscope. All images were acquired and processed in TIFF format, analysis was done using Image ProPlus 6 AMS software (Media Cybernetics Inc., Buckinghamshire, UK). The same light level as for incidental light without a slide was kept for each image acquired.

### 2.3. Western blotting analysis

Pulmonary tissues ($n = 4$ in each group) were grounded quickly on ice, 1 mL of protein splitting liquid was added per 100 mg sample and the supernatant was collected after centrifugation. The protein concentration was measured using the BCA method. The samples were separated with 40 μg of 12% SDS–PAGE (polyacrylamide gel electrophrosis). They were then transferred onto a nitrocellulose membrane. The membrane was blocked with nonfat milk for 2 h, incubated with the primary antibody for 2 h and washed four times with TBST for 5 min each time. The membrane was then incubated with the secondary antibody labeled with peroxidase for 1 h. The ECL method was used for image development. The average gray value was measured with Image J 1.42q software after scanning.

### 2.4. Statistical analysis

Data were presented as means ± the standard deviation (SD). Comparisons were made by performing paired Student's *t*-test as appropriate. Significance was determined when $P < 0.05$ was obtained.

## 3. Results

### 3.1. Histological analysis

H&E staining revealed extensive hyperplasia and hypertrophy in goblet cells in the asthma group (Fig. 1 B). They were filled with excreted mucus and exfoliated cells which formed mucus plugs (Fig. 1D). The submucosa showed evidence of infiltration of inflammatory cells together with smooth muscle thickening (Fig. 1D). High numbers of inflammatory cells, including lymphocytes and eosinophilic granulocytes were also observed in the asthma group when compared with the control group ($P < 0.00001$; Fig. 1E).

### 3.2. Distribution and expression of AQPs by immunohistochemistry and Western blotting analysis

In the small airways, AQP1 was distributed mainly in sub-epithelia MECs and surrounding connective tissue cells (Fig. 2 A). In the alveoli, AQP1 mainly localized to the alveolar MECs and red blood cells (Fig. 2C). In the asthma group, AQP1 expression was relatively low in alveolar MECs (Fig. 2D), but was relatively high in small airways sub-epithelial (Fig. 2B). AQP1 IOD observed, in asthma groups AQP1 staining intensity increased in small airways, but decreased in alveoli ($P < 0.05$; Fig. 2E). Western blotting, indicated that AQP1 expression in the asthma group was approximately 57% lower than in the control group ($P < 0.05$; Fig. 2F and G).

In control group, AQP5 was distributed mainly in the apical membranes of non-ciliated epithelial cells in small airways, ciliated cells were negative for AQP5 (Fig. 3 A). Expression of AQP5 of the small airways was higher in the asthma group than that in the control group (Fig. 3B). Positive expression of AQP5 was observed in the inner surface of alveoli in the control group, with evidence of strong expression in the apical membranes of type I alveolar epithelial cells and type II epithelial cells (Fig. 3C and D). In contrast, AQP5 expression was significantly decreased in the alveoli of the asthma group (Fig. 3E). AQP5 IOD observed, in asthma group, AQP5 staining intensity increased in small airways, but decreased in alveoli ($P < 0.05$; Fig. 2F). Western blot analysis indicates that AQP5 expression was approximately 36% lower in the in asthma group than that in the control group ($P < 0.05$; Fig. 3G and H).

## 4. Discussion

Water transfer and mucous retention in small airways and alveoli are critical steps in the pathogenesis of asthma where there are no typical gland structures. The role played by AQP1 in vascular permeability has been demonstrated in a previous study which