·Basic Research·

Role of aquaporins -1 in corneal endothelial fluid transport

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Abstract

• AIM: To investigate the expression of aquaporins-1 (AQP-1) in cultured bovine corneal endothelial cells and to explore the role of AQP-1 in corneal endothelial fluid transport.

• METHODS: The bovine corneal cells were cultured in DMEM containing 200mL/L neonate bovine serum. AQP-1 expression in the bovine corneal endothelial cells was detected with immunohistochemistry method before and after treatment of the cells with aquaporin inhibitor, *p* -chloromercuribenzene sulfonate. The osmotic water permeability was determined by monitoring volume changes of cultured bovine corneal endothelial cells.

• RESULTS: Positive staining was used to reveal the AQP-1 expression in the membrane of cultured bovine (in brown color). The reading of osmotic water permeability of the cultured bovine corneal endothelial cells before treatment with *p* -chloromercuribenzene sulfonate was 0.044 ± 0.005cm/s, which significantly decreased to 0.017 ± 003cm/s after treatment (n=15).

• CONCLUSION: AQP-1 expressed in the membrane of cultured bovine corneal endothelial cells may play an important role in fluid transport of corneal endothelial cells. Alteration of the AQP-1 expression may cause abnormal corneal function and corneal edema.

• KEYWORDS: corneal endothelium; *p*-chloromercuribenzene sulfonate; aquaporin-1

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INTRODUCTION

The cornea is the anterior tissue of the eye, and its transparency is essential to optical function. As corneal transparency requires approximately 78% water content (by weight), water entering the cornea must be extruded to maintain its natural hyperosmotic state [1]. The research of Riley's *et al* [2] shows that the corneal endothelium is responsible for at least 90% of the fluid transport of the rabbit cornea. Therefore, corneal endothelium plays a great role in maintaining corneal transparency. Up to now, the mechanism of corneal endothelial fluid transport is still unclear. Some recent studies show the presence of aquaporin-1 (AQP-1) in corneal endothelium. In the present study, we have detected the expression of AQP-1 in cultured bovine corneal endothelial cells (CBEC) by immunohistochemistry and explored the potential role of AQP-1 in corneal endothelial fluid transportation.

MATERIALS AND METHODS

Culture of Corneal Endothelial Cells The bovine corneas from a local slaughterhouse were excised under sterile conditions. The endothelial layer was covered with a 0.5g/L trypsin(0.53mmol/L EDTA-Na solution (Gibco) at 37℃ for 5 minutes. The endothelium was then scraped gently, and the dispersed cells were aspirated and transferred into DMEM (Gibco) containing 50mL/L fetal bovine serum (FBS) and 100kU/L penicillin and 100μg/L streptomycin, then incubated in the humid incubator with 50mL/L CO_2 at 37℃ and fresh culture medium was added every 2-3 days. The resultant second-passage cultures was subcultured onto coverslips and used for the experiments.

Immunohistochemistry of Cell Coverslips Cells growing on the coverslips were washed three times with D-Hank's for 2 minutes and fixed in dehydrated cool acetone for 15 minutes, followed by washing with D-Hank's for 2 minutes, 5mL/L hydrogen peroxide in 100% methanol for 30 minutes, and distilled water three times sequentially. After treatment with antigen repairing liquid for 10 minutes, the coverslips were washed twice with distilled water. After that, the coverslips were incubated with 100mL/L goat serum for 10 minutes at room temperature to block nonspecific antibody binding. Then it was incubated with the primary antibodies against AQP-1 (1:200) for 1 hour and were then incubated

with the secondary antibody (biotinylated goat anti-rabbit IgG) at a dilution of 1∶50 for 20 minutes at 37℃, then washed four times for 5 minutes with PBS, and then incubated in DAB developer solution at room temperature for 5 to 30 minutes. After dehydration and mounting, the coverslips were observed under a light microscope. The brown grains in cellular membrane indicated positive staining.

Determination of Osmotic Water Permeability (Pf) Corneal endothelial cells were divided into two groups: the experimental group treated with 1mmol/L *p*-chloromercuri-benzene sulfonate (pCMBS, osmotic water permeability) for 20 minutes, and the control group untreated. Osmotic swelling was analyzed from volume changes of the cells transferred from 200mOsm to 70mOsm MBS diluted with deionized water. The sequential cell image was photographed at 30 seconds intervals for a total of 3 minutes and the volumes of the sequential images were calculated by an image-processing system. The Pf was calculated using the following formula:

$$V = (4/3) \times (\text{area}) \times (\text{area}/\pi)^{1/2}$$

$$Pf = \frac{V_0 \times d(V/V_0)/dt}{S \times V_w \times (Osm_{in} - Osm_{out})}$$

while $V = (4/3) \times (\text{area}) \times (\text{area}/\pi)^{1/2}$

The area is the measured maximal cross sectional area of a cell; Osm_{in} is intra-cellular osmotic pressure; Osm_{out} is extra-cellular osmotic pressure; Pf is determined by the initial slope of time course of V/V_0 (d (V/V_0)/dt), the initial endothelial cell volume V_0 (cm^3), the initial cell area S(cm^2) and the molar volume of water Vw (18mol/ cm^3).

Statistical Analysis Data were presented as mean±SD and Student's *t*-test (SPSS) was used for statistical analysis. $P<0.05$ was considered to be significant.

RESULTS

The CBCECs revealed irregular shapes, including polygons, fusiforms and circles. All cells had a large and limpid cytoblast. The cellular membrane of CBCEC showed positive staining by immunohistochemistry(Figure 1), suggesting the expression of AQP-1 in the cellular membrane of corneal endothelia. The Pf of cells in the experimental group (0.017±0.003cm/s, n=15) was signi- ficantly (P=0.003) lower that that in the control group (0.044±0.005cm/s, n= 15). These results suggested that AQP-1 function in cellular membrane was inhibited by the inhibitor of osmotic water permeability.

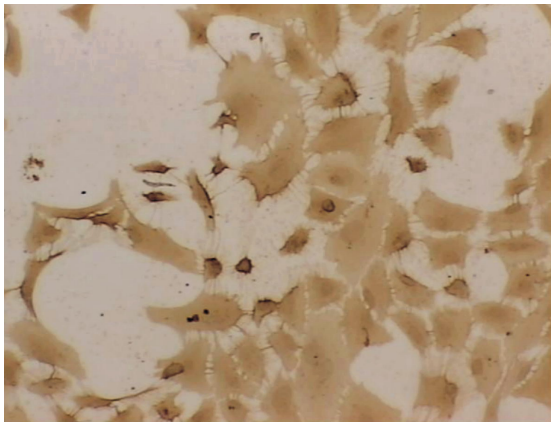

Figure 1 Positive staining (showing brown grains) of cultured bovine corneal endothelial cell membranes by anti –AQP –1 antibodies (×200)

DISCUSSION

Aquaporins (AQP) are a class of membrane proteins that are related to cell fluid transport. So far, 11 hypotypes in humans and mammals (AQP0, and AQP1-10) have been identified. The function of AQP is to mediate water, glycerine and urea transbiomembrane transport and participate in many physioprocesses [4,5]. AQP also plays important roles in several ocular tissues [6]. Rat ocular tissue contains aquaporins in the ciliary body, cornea, lens, retina, iris, and choroid with AQP-1 comprising the highest proportion [7]. In the cornea, AQP-1 is confined to the endothelium. There is no continuous tight junction between corneal endothelial cells, which causes aqueous fluid to continuously leak into the corneal stroma. Furthermore, the corneal stroma is mildly hyperosmolar in relation to the aqueous fluid of the anterior chamber, which causes fluid entering the cornea. However, we did not observe corneal edema because of increasing water content at the physiologic state. This may caused by the presence of AQP-1 in corneal endothelial cells. But the function of AQP-1 in corneal endothelial fluid transport has not been well defined.

The present study showed that the membranes of CBCECs were positively stained, as shown by the appearance of brown grains, by anti-AQP-1 antibodies, suggesting the expression of AQP-1 in BCEC membranes. Pf analysis showed that CBCECs had higher osmotic water permeability, in agreement with the previous report [8]. After treatment with pCMBS, an inhibitor of the AQP-1 water channel, the Pf of cells was obviously decreased. This finding suggests that AQP-1 plays important roles in corneal endothelial fluid transport.

Many processes (*i.e.*, trauma, disease, iatrogenics, *etc.*)

cause damage to the corneal endothelium and result in corneal edema. Pseudophakic bullous keratopathy (PBK) is a corneal disease that occurs after cataract removal and placement of an intraocular lens. When an intraocular lens is not placed in the eye during cataract surgery and there is associated corneal edema, the disease process is called aphakic bullous keratopathy (ABK). A clinical hallmark of both PBK and ABK is chronic corneal edema, which leads to significant loss of transparency in the central cornea. Fuchs' endothelial dystrophy is a hereditary disease. It is most common in older women and has many clinical features similar to PBK/ABK corneas. Abnormal fluid accumulation is a major feature of both PBK/ABK and Fuchs' dystrophy corneas, but the mechanism has not been understood yet. Kenney *et al* [9] and Elizabeth *et al* [10] have found that AQP-1 in Fuchs' endothelial dystrophy/BK corneas is lower than that in normal corneas. Therefore, abnormalities of AQP-1 may be the cause of these diseases, which may be treated by regulating the expression of AQP-1.

These results confirm that QP-1 presented in corneal endothelial cell membrane may play an important role in fluid transport in corneal endothelium. This study contributes toward understanding the mechanism of corneal fluid transport and identification of new therapeutics for treatment of corneal edema diseases.

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