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Less Invasive Corneal Transepithelial Electrical Resistance Measurement Method

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ABSTRACT **Purpose: To evaluate acute corneal permeability changes after instillation of benzalkonium chloride (BAC) using a newly developed in vivo less invasive corneal transepithelial electrical resistance (TER) measurement method in animals and humans. Methods: We previously developed an in vivo method for measuring corneal TER using intraocular electrodes in animals. This method can be used to precisely measure the decline of the corneal barrier function after instillation of BAC. To lessen the invasiveness of that procedure, we further refined the method for measuring the corneal TER by developing electrodes that could be placed on the surface of the cornea and in the conjunctival sac instead of inserting them into the anterior chamber. Corneal TER changes before and after exposure to 0.02% BAC were determined in this study using the new device in both rabbits and humans. Results: There was a significant decrease in the corneal TER after exposure of the cornea to 0.02% BAC solution in both rabbits and humans ($P<.01$). The results of this new less invasive method agreed with those of formerly established anterior chamber methods in rabbit experiments. Conclusion: This new less invasive corneal TER measurement method enables us for the first time to measure TER of the human cornea, allowing safe and reliable investigation of the direct effect of different eye drop treatments on the corneal epithelium.**

KEY WORDS **benzalkonium chloride, cornea, transepithelial electrical resistance**

I. INTRODUCTION

The cornea is one of the few human tissues that is always in direct contact with the environment. This together with its transparency makes the cornea a very special tissue. In particular, the corneal epithelium, which is the outer part of the cornea, acts as a barrier against the daily insults of the external environment. To ensure its transparency, the cornea does not have blood vessels for its nourishment. Nutrients are supplied by diffusion through the epithelium and endothelium layers, ensuring a proper homeostasis.[1] Corneal epithelium tight junctions are the most apical intercellular junctions and play an important role in the establishment and maintenance of the barrier function.[2,3] Disruption of corneal epithelial barrier function results in ocular irritation[4,5] and increased risk for microbial infections.[6]

The electrophysical properties of a cell or tissue can be determined by passing an electric current through the cell or tissue and measuring the voltage drop and potential difference across the tissue. When the current delivered and the voltage measured are known, the resistance of the tissue can be calculated using Ohm's Law: resistance (Ω) is equal to the voltage (V) divided by the current (I in amperes).[7]

Most ophthalmic drugs contain adjuvants such as surfactants and preservatives. They are often essential for ocular liquid formulations, solubilizing drugs, and preventing microbial contamination. Some of these adjuvants act as ocular penetrating enhancers, promoting drug penetration through the strong corneal barrier and modifying the physiochemical property of drugs.[8,9] At the same time, however, they damage the corneal epithelial structure, especially the transcellular integration of superficial cells, which is mainly maintained by tight junctions.[10,11] Therefore,

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OUTLINE

investigation of the effect on the cornea of ophthalmic drugs and adjuvants is important.

Corneal transepithelial electrical resistance (**TER**) is maintained by corneal superficial cells with tight junctions between them, which together function as a corneal barrier that is highly resistant against invasion. Measuring changes in TER allows the quantitative and continuous evaluation of the state of the corneal epithelium and its barrier function. The method is used for electrophysiologically evaluating corneal toxicity induced by ophthalmic drugs.[12,13]

Measurement of corneal TER is a suitable method for evaluating corneal permeability and irritation quantitatively and continuously. TER reflects the barrier function of the epithelium. Lower corneal TER means more electrical current penetrates through the damaged superficial cells and tight junctions between them. In addition, it is reported to be a very sensitive test for measuring electrical properties of the cornea.[14,15]

In vitro experimental systems using cultured cells are often employed to evaluate the barrier function of the corneal epithelium as well as injuries of the corneal epithelium caused by various drugs.[7,16,17] These experimental systems provide an excellent means of objectively comparing the potential for corneal injury among several ophthalmic solutions; however, the extent to which these experimental models reflect the condition of the eyes in vivo remains unclear.[18]

Only a few in vivo studies of corneal electrical properties have been reported,[19-21] due to the difficulty of performing such analyses. Recent approaches to performing in vivo measurements have adapted the existing TER measurement methods for use in living animals.[7,22]

We developed a new method of measuring the TER of live rabbit cornea. In this method, the cornea is not damaged by the experimental procedure and the TER is stable before drug administration. To measure corneal TER, we used a volt-ohm meter which generates ± 20 μA AC square wave current at 12.5 Hz. Therefore, it could measure TER every 0.08 s. In addition, TER was monitored with a recorder, which shows TER changes continuously. To our knowledge, no other study has evaluated acute corneal change within one second in vivo.[23]

However, the invasiveness of this procedure precludes its use in clinical practice. In order to overcome this problem, we recently developed a less invasive corneal TER measurement method.[24]

In previous studies, after developing a new in vivo method of measuring the TER of rabbit corneas, we demonstrated that benzalkonium chloride (**BAC**) concentrations between 0.005% and 0.02% immediately caused acute corneal barrier dysfunction.[23,25] The purpose of this study was to evaluate acute corneal permeability changes after instillation of BAC using a new less invasive in vivo TER measurement method in animals and humans.

II. MATERIALS AND METHODS

A. Chemicals

Ca^{2+}- and Mg^{2+}-free Hank's Balanced Salt Solution (**HBSS**) was obtained from Invitrogen Corporation (Carlsbad, CA, USA). BAC 10% solution (mixed BAC) was obtained from Wako Pure Chemical, Co, (Osaka, Japan).

B. Experimental Animals

Male white Japanese rabbits (KBT Oriental, Tosu, Japan) weighing 2.5 -3.0 kg were individually housed in cages under a controlled temperature (21°C) and humidity (50 ± 5%) and a 12:12 h light/dark cycle at the Laboratory Animal Center for Biomedical Research, Nagasaki University School of Medicine. The study was initiated when the rabbits reached weights of 3.0-4.0 kg, as this was the point where the corneal diameters were of suitable size for experimentation. The rabbits were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

C. Corneal Transepithelial Electrical Resistance Measurement In Vivo in Rabbits

The rabbits were anesthetized with an intramuscular injection of 30 mg/kg ketamine (Ketalar, Sankyo, Tokyo, Japan) and 5 mg/kg xylazine (Celactal, Bayer HealthCare, Osaka, Japan). A 1.0-mm diameter custom-made Ag/AgCl electrode (Physiotech, Tokyo, Japan) was placed within the tear fluid in the conjunctival sac. The pathway of the electrical current is shown in Figure 1. A 6.0 mm internal diameter (0.28 cm^2 inner area) nitrile rubber O-ring (Union Packing, SAN-EI, Osaka, Japan) was fixed on the cornea using biomedical adhesive (Alon-Alpha A, Sankyo, Tokyo, Japan). Subsequently, 80 μL of HBSS was placed inside the ring, with the second electrode then placed in HBSS on the cornea. In a period of just a few seconds, 1 mL of the HBSS as a control and 0.02% BAC were gently poured into the ring, with overflow aspirated. After an exposure period of 30 seconds, the rings were washed out with 1 mL of HBSS.