Specular Microscopy of Corneal Endothelial Cells in Cynomolgus Monkeys
Haruo MORITA, Kazuhiro SHIMOMURA, and Yoshito SAKUMA
Preclinical Research Division, Central Institute for Experimental Animals, 1433 Nogawa, Miyamae-ku, Kawasaki 216, Japan
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ABSTRACT. The corneal endothelia of 15 male and 16 female cynomolgus monkeys (estimated age: 2 years and more) were examined with a contact-type specular microscope under general anesthesia. The specular microscopy showed uniform-sized, hexagonal endothelia arranged regularly on the innermost layer of the cornea. Concerning the cell density (cells/mm²), no statistically significant difference was observed between the left and right eyes or between sexes. However, the cell density decreased in monkeys over 7 years old. —KEY WORDS: corneal endothelium, cynomolgus monkey, specular microscopy.

A corneal endothelial layer, located between the Descemet membrane and the anterior chamber, plays an important role in maintaining the transparency of the cornea [4]. Clinical specular microscopy of the cornea is commonly conducted in cases such as cataract extraction, intraocular lens implantation, and transplantation of corneas in modern ophthalmology. But, except for a small amount of data in fundamental medicine [1, 3, 5], there has been little work reported on specular microscopy in veterinary medicine. The purpose of this study was to obtain basic ophthalmological data of specular microscopy to be used in monkey toxicity studies.

Fifteen male and 16 female clinically healthy cynomolgus monkeys (Macaca fascicularis) from Indonesia were used in this study. Estimated age of the 31 animals was 2 years and more. Body weights ranged from 2.5 to 5.3 kg at the time of the ophthalmological examination. All animals were housed in individual cages in an animal room maintained at a temperature of 23–27°C and a relative humidity of 40–80%. Each animal was given 100 g of monkey chow (CMK-1, CLEA Japan, Inc.) daily and tap water ad libitum.

Prior to the examination, combination of 10 mg/kg of ketamine hydrochloride (Ketalar, Sankyo & Parke-Davis Co., Inc., Japan) and 1.0 mg/kg of xylazine hydrochloride (Celactar, Bayer, Germany) was intramuscularly administered for general anesthesia, and 0.4% oxybuprocaine hydrochloride (Benoxil, Santen Pharmaceutical Co., Ltd., Japan) was instilled on the cornea for local anesthesia. The observation of both eyes was performed on each monkey using a contact-type specular microscope (Model SP-5500, Konan Kamera Res., Inc., Japan) with a cone lens (× 40). After observing and photographing the central corneal endothelium, numbers of the corneal endothelial cells per unit area (cell density, cells/mm²) were counted five times on enlarged photographs (× 200) by the Fix frame method [6] and averaged. Statistical analysis was performed by Student’s t-test to compare the cell density between the left and right eyes and between sexes, and by one-way analysis of variance to compare the data among different age groups. Difference was considered statistically significant if the P value was less than 0.05. Comparison was also performed between one group of the animals over 7 years old and those of 2, 3, 4, 5 and 7 years old animals by the least significance difference (L.S.D.) method [2].

Fig. 1. Specular microscopy of the corneal endothelium of a male monkey (5 years old). Hexagonal endothelia are tightly packed together. × 100.

Fig. 2. Relationship between the corneal endothelial cell density and aging. The cell density declined in monkeys over 7 years old. *: Significantly different (p<0.05) from 2, 3, 4, 5 and 7 years old groups.
Table 1. Corneal endothelial cell density (cells/mm²) of monkeys

<table>
<thead>
<tr>
<th>(cells/mm²)</th>
<th>Male</th>
<th>Female</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>R a)</td>
<td>L b)</td>
</tr>
<tr>
<td>Mean</td>
<td>3653</td>
<td>3712</td>
</tr>
<tr>
<td>S.D. c)</td>
<td>233</td>
<td>307</td>
</tr>
<tr>
<td>Max. d)</td>
<td>3690</td>
<td>4460</td>
</tr>
<tr>
<td>Min. e)</td>
<td>3280</td>
<td>3160</td>
</tr>
<tr>
<td>95% C.L. f)</td>
<td>3520-3787</td>
<td>3536-3888</td>
</tr>
<tr>
<td>Number of</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age g)</td>
<td>2-5</td>
<td>4-7&lt;</td>
</tr>
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</table>

a) Right, b) Left, c) Standard deviation, d) Maximum, e) Minimum, f) 95% confidence limits, g) Years old.

Specular microscopy allowed corneal endothelial examination in both morphological and numerical aspects. The endothelial cells were hexagonal in shape and tightly packed together (Fig. 1). They were relatively uniform in size and regularly arranged throughout the entire cornea. The characteristic features of the endothelium were well maintained in all the animals examined and there were no discernible morphological differences between sexes or among different age groups. Concerning the cell density (cells/mm²), no statistically significant difference was observed between the left and right eyes or between sexes (Table 1). However, there was an age-associated decrease in the cell density. The mean cell density in the group of animals over 7 years old, which was the lowest among all age groups, differed significantly from those of 2, 3, 4, 5 and 7 years old animals (Fig. 2). The mean cell density in the other age groups was comparable. These findings were consistent with those reported in humans; the corneal endothelial cells in the left and right eyes and in males and females are similar in density [6], which decreases with age [6]. In humans, the corneal endothelial cells vary in size and decrease in the number of hexagonal cell with age [6]. In our material, however, all aged groups including the oldest one, were similar endothelial morphology.

REFERENCES