Specular Microscopy of the Corneal Endothelial Cells in Common Marmosets

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ABSTRACT. The corneal endothelia of 12 female common marmosets (age: 12–18 months) 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. The endothelial plane was smooth; however, it occasionally had irregular undulation or parallel folds. The cell density (cells/mm²) in common marmosets was similar to that in cynomolgus monkeys. — **KEY WORDS:** common marmoset, corneal endothelium, specular microscopy.

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The corneal endothelial layer, located between the Descemet's membrane and the anterior chamber, plays an important role in maintaining the transparency of the cornea [14]. This densely packed monolayer presents a permeability barrier to leakage of the aqueous humor into the clear stroma of the cornea, which would lead to edema and opacification of the cornea. 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 [3, 4, 9, 15], there have been few works reported on specular microscopy in veterinary medicine. The purpose of this study was to obtain basic ophthalmological data on specular microscopy to be used in ocular toxicity studies.

Twelve clinically healthy female common marmosets (*Callithrix jacchus*), which were born at our institute, were used in this study. The age of the animals was from 12 to 18 months and body weights ranged from 270 to 420 g. All animals were caged in pairs and kept in an animal room maintained at a temperature of 25–28°C and a relative humidity of 50–70% with a 12-hr light/dark cycle. Each animal was given daily about 40 g of New World monkey chow (Oriental Yeast, Co., Ltd.) supplemented with a bit of vitamin C and D3, chicken meat and dried milk, and tap water *ad libitum*.

Prior to the examination, a combination of 25 mg/kg of ketamine hydrochloride (Ketalar, Sankyo & Parke-Davis Co., Inc., Japan) and 2.0 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.

Both eyes of each animal were examined using a contact-type specular microscope (Model SP-5500, Konan Camera Res., Inc., Japan) with a cone lens (× 40). Numbers of the corneal endothelial cells per unit area (cell density, cells/mm²) were counted five times by the fixed frame method [16] on enlarged photographs (× 200) taken from the central cornea, and averaged. Prior to the specular microscopy, the anterior segment of both eyes were examined with a slit-lamp biomicroscope (Model SL-2, Kowa, Japan) and the

fundus with a fundus-camera (Model RC-2, Kowa, Japan). A few drops of 0.5% tropicamide and 0.5% phenylephrine (Midrin P, Santen Pharmaceutical Co., Ltd., Japan) was applied as a mydriatic. Statistical analysis was performed by Student's *t* test to compare the cell density between the left and right eyes. Statistically significant difference was assumed if *P* value was less than 0.05.

Specular microscopy allowed morphological and numerical examination of the corneal endothelial cells. The endothelia were hexagonal in shape and packed tightly together (Fig. 1). They were relatively uniform in size and regularly arranged throughout the entire cornea. The endothelial plane was smooth; however, in some cases, it had irregular undulation (Fig. 1) or parallel folds (Fig. 2). Concerning the cell density (cells/mm²), no statistically significant difference was observed between the left and right eyes (Table 1). Examinations with a slit-lamp biomicroscope and a fundus-camera revealed no ocular abnormalities in any animals.

In toxicological research, monkeys have been commonly used as one of non-rodent species. However, because of recent restrictions on export of wild-caught monkeys from their natural habitat, the monkeys for research use are becoming unavailable. Common marmosets (*Callithrix jacchus*) have been developed as experimental animals, and are increasingly used in several different fields of biomedical research [7] because of their unique biologic characteristics, small size, relatively low procurement and maintenance costs, ease of handling, and reproductive capability in captivity [7, 17]. Ophthalmological data of common marmosets have been reported [1, 2, 10, 18] and indicate some similarities to those of macaques [2, 11–13, 18]. To the best of our knowledge, however, no reports on specular microscopy in common marmosets have been published. Therefore, the present study was conducted in order to obtain basic ophthalmological data on specular microscopy to be used in ocular toxicity studies. Recent development of a specular microscope allows direct *in vivo* visualization of the corneal endothelia. However, specular microscopy has rarely been applied to ophthalmological examinations in toxicity studies. We have already examined the corneal endothelia of cynomolgus monkeys [5] and rabbits [6] with a contact-type specular microscope. The endothelial cells

Fig. 1. Specular microscopy of the corneal endothelia of a common marmoset. Hexagonal endothelia are tightly packed together. Irregular undulation is evident ($\times 200$).

Fig. 2. Specular microscopy of the corneal endothelia of a common marmoset. Parallel folds are observed ($\times 200$).

of common marmosets were hexagonal in shape and tightly packed together, which was very similar to the findings obtained previously from cynomolgus monkeys. Among hexagonal endothelial cells, large cells were scattered in rabbits [6]. The endothelial cells of common marmosets

were uniform in size and no such large cells were observed. The endothelial plane was usually flat in cynomolgus monkeys and rabbits. In contrast, the plane occasionally had undulation and parallel folds in common marmosets. The similar endothelial folds were infrequently found in

Table 1. Corneal endothelial cell density (cells/mm²) of common marmosets

(cell/mm ²)	Right	Left
Mean	3767	3575
S.D. ^{a)}	239	350
Max. ^{b)}	4200	4000
Min. ^{c)}	3350	2900
95% C.L. ^{d)}	3615–3918	3353–3797
Number of animals	12	
Age ^{e)}	12–18	

a) Standard deviation

b) Maximum

c) Minimum

d) 95 % confidence limits

e) Months old

cynomolgus monkeys in our laboratory, and have been reported in humans as posterior corneal rings which is considered to be attributable to the mechanical pressure of the cone lens on the cornea [8]. No discernible ocular abnormalities were associated with these endothelial findings in common marmosets, and the undulation and folds are also thought to be the result of unavoidable pressure by the cone lens rather than endothelial structural variation. Care must be taken to distinguish these artifactitious findings from endothelial lesions. The folds can be reproduced and utilized as an anatomical landmark to relocate the field of examination [8]. Concerning cell density (cells/mm²), no statistically significant difference was observed between the left and right eyes. These findings were consistent with those reported in cynomolgus monkeys [5], rabbits [6], and human [16] where the density of corneal endothelial cells is similar in the left and right eyes. The mean cell density in common marmosets was 3,575 to 3,767 (cells/mm²) and similar to that reported in cynomolgus monkeys (3,500 to 3,700 cells/mm²). But it was greater than in human (2,700 to 3,500 cells/mm²) and in rabbits (2,695 to 3,224 cells/mm²). The cell density varies with age [5, 6, 16], and the difference between common marmosets and humans, and

rabbits may be the results of different age groups from which the data were obtained in addition to interspecies variation.

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