Preparation of Ophthalmic Formulations Containing Cilostazol as an Anti-glaucoma Agent and Improvement in Its Permeability through the Rabbit Cornea

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Abstract: To evaluate the pharmacological properties of cilostazol (CLZ), we examined its intraocular pressure (IOP)-lowering effect. CLZ is an inhibitor of Type III phosphodiesterase that increases intracellular cyclic AMP levels by restraining platelet aggregation, and has a potential protective effect against atherosclerosis. We attempted to apply it for use as an anti-glaucoma agent; however, the application of CLZ in the ophthalmic field is limited due to its poor water solubility. We attempted to enhance CLZ solubility using 2-hydroxypropyl-β-cyclodextrin (HPβCD). The solubility of CLZ increased with increasing HPβCD concentrations, and 0.05% CLZ was dissolved in 10% HPβCD. Moreover, fine particle suspension of 0.5% CLZ in 5% HPβCD (soluble CLZ: ca. 0.027%) were prepared using a Microfluidizer, an impact-type emulsifying comminution device. In an in vitro transcorneal penetration experiment through the rabbit cornea, the CLZ penetration rate was dependent on the CLZ content of the solutions and suspensions. When a 0.05% CLZ ophthalmic solution was instilled into a rabbit eye, the absorption rate constant for CLZ into an aqueous humor was 0.0059 ± 0.001 min⁻¹, and the elimination rate constant was 0.048 ± 0.024 min⁻¹. Also CLZ ophthalmic solutions and fine particle suspension were examined for their ability to reduce enhanced intraocular pressure (IOP) of rabbits in a darkroom. The instillation of 0.05% CLZ ophthalmic solutions and 0.5% CLZ fine particle suspensions into rabbit eyes reduced the enhanced IOP. These results demonstrate that the instillation of CLZ ophthalmic solutions and fine particle suspensions may represent an effective anti-glaucoma formulation.

Key words: glaucoma, cilostazol, intraocular pressure, 2-hydroxypropyl-β-cyclodextrin, eye drops

1 INTRODUCTION

Glaucoma is characterized as nervous degeneration that causes the disappearance of retinal ganglion cells, visual field loss, and excavation of the optic disk and ophthalmopathy. Intraocular pressure (IOP) in the eye is regulated to remain within a normal range. In abnormal situations such as in glaucoma, the IOP is often elevated, causing damage to the optic nerve head that leads to blindness. Because glaucoma is the second leading cause of blindness in humans, extensive efforts have been made to develop antiglaucoma drugs and laser and other surgical procedures to lower IOP. Most of these modalities are intended either to modulate the facility of aqueous outflow at sites of the trabecular meshwork (TM) and ciliary muscle (CM), or to inhibit aqueous humor production by the ciliary body. Despite the widely recognized functional importance of TM and CM tissues in regulating aqueous outflow, the cellular mechanisms underlying these functions are not well understood. Therefore, the IOP caused damage to the retina and nerve are not satisfactorily controlled by current drug therapies.

Rho guanosine triphosphatase (GTPase), a member of a subgroup of the Ras superfamily, participates in signaling pathways that lead to the formation of action stress fibers and focal adhesions. Rho is also involved in diverse physiological functions associated with cytoskeletal rear-
rangement, such as cell morphological functions, cell motility, cytokinesis, and smooth muscle contraction. Recently, several putative target molecules of Rho have been identified as Rho effectors, including Rho-associated coiled coilforming protein kinase, referred to as p160ROCK, and its isoform, ROKα/Rho kinase/ROCK II (ROCKs). ROCK has been shown to be expressed in ocular tissues, including TM and CM\(^4\). Honjo et al. reported that the instillation of Y-27632, a selective ROCK inhibitor, significantly reduces IOP, the mechanism for which was attributed to improved outflow\(^4\). Inhibition of ROCK activity has been shown to induce alterations in TM cellular responses such as migration, adhesion, and changes in cell shape.

Cilostazol (6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydrocarbostyril, CLZ) is well known to have antiplatelet aggregation and vasodilatory effects with minimal cardiac effects and has been applied clinically to cerebrovascular diseases. Pharmacologically, cilostazol has been found to increase intracellular cyclic AMP levels by inhibiting its hydrolysis by type 3 phosphodiesterase\(^2\). Therefore, if CLZ possesses inhibitory activity against ROCK in TM cells, CLZ may be able to reduce IOP.

In the present study, we examined the ability of CLZ on to reduce the enhanced IOP of normal rabbits in a darkroom. However, the application of CLZ in the ophthalmic field is limited due to its poor water solubility. Therefore, we attempted to enhance CLZ solubility using 2-hydroxypropyl-β-cyclodextrin (HPβCD). Also we tried to improve the transcorneal penetration ability of CLZ using fine particle formation with a Microfluidizer, an impact-type emulsifying comminution device.

2 EXPERIMENTAL

2.1 Animals

Male Japanese white rabbits, 2.5–3.0 kg, were used in this study (SLC Inc., Shizuoka, Japan). They were housed under standard conditions (12 h/day fluorescent light (07:00–19:00), 25 ± 1°C) and allowed free access to a commercial diet (CR-3, Clea Japan Inc., Tokyo) and water. All procedures were performed in accordance with the Kinki University School of Pharmacy Committee for the Care and Use of Laboratory Animals.

2.2 Materials

CLZ was kindly donated by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Low-substituted methylcellulose (METLOSE SM-4, MC) was provided by Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Benzalkonium chloride was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Y-27632 was purchased from Wako Pure Chemical Industries (Osaka, Japan), and 0.4% Benoxil ophthalmic solution was provided by Santen Pharmaceutical Co., Ltd. (Osaka, Japan). All other chemicals used were of the highest purity commercially available.

2.3 CLZ solubility studies

Studies were carried out using two different methods: equilibration and solid addition methods. Briefly, in the equilibration method, 50 mg of CLZ was dispersed in 10 mL of HPβCD solution (5, 10, 15 and 20 %, w/v) dissolved in saline. The mixtures were stirred for 24 h under cover at room temperature, and then filtered through Minisart CE (pore size of 0.20 μm, Sartrius Biotech GmbH, Goettingen, Germany). In the solid addition method, 50 mg of CLZ solid powder was dispersed in 8 mL of saline, and then 0.5, 1.0 and 2.0 g of solid HPβCD was added gradually within 10 min into the dispersion at room temperature. After stirring for 30 min longer, the mixtures were adjusted to 10 mL with saline and filtered through Minisart CE (pore size of 0.20 μm). The amount of CLZ in the filtrates was determined by the following HPLC method. Five microliters of filtrate was added into 50 μL methanol containing 0.25 μg indomethacin (internal standard), and 10 μL of this solution was injected into a Mightysil RP-18 (3 μm, column size: 2.0 mm × 50 mm) column (Kanto Chemical Co., Inc., Tokyo, Japan) using a Shimadzu LC-10AD system equipped with a CTO-6A column oven (Shimadzu Corp., Kyoto, Japan). The mobile phase consisted of acetonitrile/methanol/water (35/15/50, v/v/v). The flow rate was 0.25 mL/min, the column temperature was 35°C, and the wavelength for detection was 254 nm.

2.4 Preparation of CLZ ophthalmic solutions and CLZ fine particle suspension

The 0.05% CLZ ophthalmic solution was prepared by the solid addition method. Briefly, 50 mg of CLZ solid powder was dispersed in 40 mL of saline containing 0.005% benzalkonium chloride (as a preservative), and then 10 g of solid HPβCD was added gradually within 10 min to the dispersion at room temperature. After stirring for an additional 30 min, the mixture was adjusted to 50 mL with saline containing 0.005% benzalkonium chloride and filtered through Minisart CE (0.10% CLZ ophthalmic solution). The 0.05% and 0.25% CLZ ophthalmic solutions were prepared by 2- and 4-fold dilution, respectively, of the 0.1% CLZ solution with saline containing 0.005% benzalkonium chloride. The 0.5% CLZ fine particle suspension was prepared using the Microfluidizer M-110-E/H (Mizuho Industrial Co., Ltd., Osaka, Japan) impact-type emulsifying comminution device. Briefly, 1.5 g of solid CLZ was suspended in 300 mL of saline containing 5.0% HPβCD, 0.5% MC and 0.005% benzalkonium chloride, and then passed 4 times through the Microfluidizer under a compressed pressure of 165 MPa, with the flow path cooled from the out-
side. The particle size in the fine particle suspension was 1.16 ± 0.76 μm (mean ± S.D.) as analyzed by a nanotrac particle analyzer UPA-EX150 (NIKKISO Co., Ltd., Tokyo, Japan); particles less than 400 nm in accounted for 9.7% of the total particles in suspension. A particle-free solution was prepared by centrifuging the 0.5% CLZ fine particle suspension at 20,400 × g for 2.5 h. The CLZ concentration in the supernatant (CLZ particle-free solution) was 0.027% as measured by the HPLC method described above.

The compositions of these CLZ ophthalmic solutions and fine particle suspensions are shown in Table 1.

### 2.5 In vitro transcorneal penetration of CLZ from CLZ ophthalmic solutions and fine particle suspension

The in vitro transcorneal penetration studies were carried out as described by Iwata et al. Rabbits were killed by injecting a lethal dose of pentobarbital into the marginal ear vein. The eyes were removed and the corneas were carefully separated from other ocular tissues. The individual corneas were placed in a methacrylate cell designed for transcorneal penetration studies. The donor chamber with the exterior surface of the cornea was filled with 3.0 mL of the CLZ ophthalmic solutions or fine particle suspension. The receiver chamber was filled with 3.0 mL of 10 mM HEPES buffer (pH 7.4) containing 136.2 mM NaCl, 5.3 mM KCl, 1.0 mM KH2PO4, 1.7 mM CaCl2 and 5.5 mM glucose. The experiments were performed at 35°C and lasted for 6 h. Five microliters of the sample solution was withdrawn from the receiver chamber at the indicated time intervals and replaced into the same volume of buffer. The CLZ concentrations were determined by HPLC as described above. Corneal viability was monitored by measuring thickness or weight (no significant changes in thickness or weight were observed over the 6 h period). The obtained data were analyzed by following equations:

\[ J_c = \frac{K_a \cdot D \cdot C_{CLZ}}{\delta} = K_p \cdot C_{CLZ} \]  
(1)

\[ \tau = \frac{\delta^2}{6D} \]  
(2)

\[ Q_t = J_c \cdot A \cdot (t - \tau) \]  
(3)

where \( J_c \) is the CLZ penetration rate, \( K_a \) is cornea/preparation partition coefficient, \( D \) is the diffusion constant within the cornea, \( C_{CLZ} \) is the CLZ content in the ophthalmic preparation, \( \tau \) is the lag time, \( \delta \) is thickness of the cornea (0.0625 cm, average of 4 rabbits), \( Q_t \) is the total amount of CLZ appearing in the reservoir solution at time \( t \), and \( A \) is the effective area of the cornea (0.78 cm²). \( J_c \) and \( \tau \) were estimated by fitting each penetration profile to Eq. 3. The penetration coefficient through the cornea, \( K_p \), is given by \( J_c/C_{CLZ} \). A nonlinear least-squares computer program was employed for the calculation.

### 2.6 In vivo transcorneal absorption of CLZ from CLZ solutions and fine particle suspension

The in vivo transcorneal absorption studies were carried out as described by Meisner et al. Rabbits were anesthetized by pentobarbital (38.9 mg/kg) injected into the marginal ear vein, and 0.4% Benoxil ophthalmic solution as a surface anesthesia agent was dropped into the eye. A 29 gauge injection needle connected to silicon tubing (Ficon SH No.00, I.D. 0.5 mm, Fuji Systems Co., Ltd., Tokyo, Japan) was inserted into the anterior chamber of the eye. Then, 50 μL of a CLZ solution or suspension was instilled into one side of the eye and 5 μL of aqueous humor was obtained through the silicon canula. The CLZ concentrations in the aqueous humor were determined by HPLC as described above.

The CLZ concentration data in the aqueous humor after a single injection of 20 μL of 0.05% CLZ solution into the anterior chamber of the eye was analyzed by Eq. 4:

\[ C_{all} = C_0 \cdot e^{-k_a \cdot t} \]  
(4)

where \( C_{all} \) is the CLZ concentration in the aqueous humor at time \( t \), \( C_0 \) is the initial concentration of CLZ in the aqueous humor, \( k_a \) is the elimination rate constant of CLZ from aqueous humor. The \( k_a \) obtained in 4 experiments was 0.048 ± 0.024 min⁻¹.

The CLZ concentration data in the aqueous humor after the instillation of 50 μL of CLZ eye drops into the eye was analyzed by Eq. 5:

\[ C_{all} = \frac{k_i \cdot F \cdot X}{V_d \cdot (k_a - k_i)} \left( e^{-k_i \cdot (t - \tau)} - e^{-k_a \cdot (t - \tau)} \right) \]  
(5)

where \( C_{all} \) is the CLZ concentration in the aqueous humor, \( X \) is the dose of the CLZ instillation, \( k_i \) is the absorption rate constant, \( V_d \) is the distribution volume (anterior chamber).
ber, ca. 150 μL), \( F \) is the fraction of CLZ absorption, and \( \tau \) is the lag time.

2.7 IOP measurement
Male rabbits were kept in a darkroom for 5 h before the experiment. A calibrated tonometer TonoPen XL (Medtronic SOLAN, FL, USA) was used to monitor the IOP after surface anesthesia (0.4% Benoxil ophthalmic solution) instilled into the rabbit eye. The 0.1% Y-27632 ophthalmic solution as a reference drug was prepared by dissolving 1 mg of it in 1.0 mL of saline. The IOPs were monitored at intervals of 5 ~ 15 min for 60 min after the instillation of 50 μL of 0.05% CLZ ophthalmic solution, intervals of 20 ~ 60 min for 180 min after the instillation of 50 μL of 0.5% CLZ fine particle suspension, and intervals of 30 ~ 60 min for 300 min after the instillation of 50 μL of 0.1% Y-27632 solution. Differences in the IOPs (\( \Delta \)IOP, mmHg) among rabbit receiving different preparations of drugs and saline were measured. The area under the curve for \( \Delta \)IOP (\( \text{AUC}_{\Delta \text{IOP}} \), mmHg · min) was calculated by following equation 6:

\[
\text{AUC}_{\Delta \text{IOP}} = \int_0^t \Delta \text{IOP} \, dt
\]  

Briefly, \( \text{AUC} \) was determined according to the trapezoidal rule up to the last IOP measurement point.

2.8 Statistics
Statistical analyses were performed using the JAM ver. 5.1 (SAS Institute Japan) computer program. Multiple groups were evaluated by one-way analysis of variance followed by Dunnett’s multiple comparison. \( p \) values less than 0.05 were considered statistically significant. The number of experiments performed in duplicate is given under the figures or tables.

3 RESULTS
3.1 Effect of HPβCD concentration on CLZ solubility
The phase solubility diagrams of CLZ in HPβCD solutions (0 ~ 20%) at room temperature are shown (Fig. 1). By the solid addition method, the increase in CLZ solubility with HPβCD concentration is linear as illustrated by the slope 7.25 mg of CLZ/g of HPβCD. On the other hand, the solubility curve for the equilibration method shows a parabolic shape; in particular, the solubility of CLZ between 5 and 15% HPβCD was lower than by the solid addition method. Therefore, the solid addition method was used for the preparation of 0.05% CLZ ophthalmic solutions.

3.2 In vitro transcorneal penetration of CLZ from CLZ ophthalmic solutions and fine particle suspension
In the \textit{in vitro} transcorneal penetration experiment, the amount of penetrated CLZ increased linearly for 6 h after the addition of all preparations into the donor chambers

\[\text{Fig. 1 Solubility Curve of CLZ in Aqueous Solutions Containing Various Concentrations of HPβCD.}\
\]

\( \text{\textbullet} \): equilibration method; \( \text{\textcircled{O}} \): solid addition method.

\[\text{Fig. 2 In Vitro Transcorneal Penetration of CLZ from CLZ Ophthalmic Solutions and CLZ Fine Particle Suspension through Rabbit Cornea.}\
\]

\( \text{\textbullet} \): 0.025% CLZ ophthalmic solution; \( \text{\textcircled{O}} \): 0.05% CLZ ophthalmic solution; \( \text{\textcircled{□}} \): 0.5% CLZ fine particle suspension, \( \square \): 0.027% CLZ particle-free solution. Each point represents the mean ± S.D. of 5 independent experiments.

(Fig. 2). The highest penetration rate \( J \) was found for the CLZ fine particle suspension, and, in order, the 0.027% CLZ particle-free solution, the 0.05% CLZ ophthalmic solution and the 0.025% CLZ ophthalmic solution (Table 2). The lag times \( \tau \) for the 0.027% CLZ particle-free solution and the 0.5% CLZ fine particle suspension were shorter than those for the 0.05 and 0.025% CLZ ophthalmic solutions. The diffusion constant \( D \) in the 0.027% CLZ particle-free solution was 2 ~ 3 times higher than that of other preparations.
3.3 In Vivo transcorneal absorption of CLZ from CLZ ophthalmic solutions and fine particle suspension

Figure 3 shows the fate of CLZ after the injection of 0.05% CLZ solution into the anterior chamber of rabbit eyes. The elimination rate constant $k_e$ was $0.048 \pm 0.024 \text{ min}^{-1}$. This value was inserted into Eq. 5 and used to calculate parameters such as $k_a$ and $F$ in the in vivo transcorneal absorption study. The CLZ concentration reached a peak at about 40 min after the installation of the 0.05% CLZ ophthalmic solutions, whereas the peak appeared at about 60 min after the installation of the 0.5% CLZ fine particle suspensions (Fig. 4). Table 3 shows the pharmacokinetic parameters for the in vivo transcorneal absorption of CLZ after the instillation of 0.05% CLZ ophthalmic solutions and 0.5% CLZ fine particle suspension into rabbit eyes. The amount of CLZ absorption $AUC$ for the 0.5% CLZ fine particle suspension was 3.3-fold higher than that for the 0.05% CLZ ophthalmic solution. However, the absorption fraction of the 0.05% CLZ ophthalmic solution ($F$: 3.3) was 3.3-fold that of the 0.5% CLZ fine particle suspension ($F$: 0.99). The absorption rate constant $k_a$ was similar for the two preparations.

3.4 Effect of the instillation of CLZ ophthalmic solutions and fine particle suspension on enhanced IOP in rabbits

IOP enhancement in rabbits was induced by keeping them in a darkroom. The IOP of rabbits kept for 3 h in a darkroom was elevated 6.3~9.7 mmHg as compared with untreated rabbits (16.9 mmHg). The elevated IOP was maintained for at least 8 h in the darkroom. Figure 5 shows the effects of 0.05% CLZ ophthalmic solutions, 0.5% CLZ fine particle suspension and 0.1% Y-27632 ophthalmic solution on reducing the enhanced IOPs of rabbits. In a simultaneous experiment using this high-IOP model rabbit, a 0.1% Y-27632 ophthalmic solution (effective time for reducing IOP: approximately 240 min) reduced the enhanced IOP longer than the 0.05% CLZ ophthalmic solution (ca. 60 min) or the 0.5% fine particle suspension (ca. 180 min). Figure 6 shows $AUC$ for $\Delta$IOP after the instillation of the 0.05% CLZ ophthalmic solution, 0.5% CLZ fine particle suspension and 0.1% Y-27632 ophthalmic solution. The $AUC$ represents the total effectiveness of the drug formulation, and the results suggest that the effectiveness of the 0.5% CLZ fine particle suspension and the 0.1% Y-27632
ophthalmic solution is approximately equal.

4 DISCUSSION

CLZ is an inhibitor of Type III phosphodiesterase that increases intracellular cyclic AMP levels by restraining platelet aggregation, and has potential protective effects against atherosclerosis. Therefore, we investigated CLZ as a possible anti-glaucoma agent. However, its application in the ophthalmic field is limited due to its poor water solubility. We attempted to enhance CLZ solubility using HP βCD by two different solubilization methods: such as an equilibration method and a solid addition method. The solubility of CLZ in low HP βCD concentrations (~5.0 μg/mL) achieved by the solid addition method was higher than that achieved by the equilibration method. It is thought the addition of solid HP βCD into water produces high local concentrations that produce a wetting environment around the surface of the CLZ solid particles thus enhancing its CLZ solubilizing power.

The 0.5% CLZ fine particle suspension showed approximately 5 times the in vitro penetration rate J and 3 times the AUC in the in vivo absorption experiment in comparison with the 0.05% CLZ ophthalmic solution. However, in the in vivo absorption experiment the absorbed fraction of the 0.05% CLZ ophthalmic solution (F: 4.9%) was 2.5-fold higher than that of the 0.5% CLZ fine particle suspension (F: 1.9%). It has been reported that particles smaller than 400 nm are absorbed by endocytosis on the ocular surface. Considering this fact, the recalculated F value of the 0.5% CLZ fine particle suspension that takes into account the effective content for absorption in the suspension (total 0.077% CLZ; 0.05% CLZ in particles less than

Table 3 Pharmacokinetic Parameters for In Vivo Transcorneal Absorption of CLZ after Instillation of 0.05% CLZ Ophthalmic Solution and 0.5% CLZ Fine Particle Suspension in Rabbit Eyes

<table>
<thead>
<tr>
<th>Formulation</th>
<th>k_{a} (min⁻¹)</th>
<th>AUC (μg · min/mL)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05% CLZ ophthalmic solution</td>
<td>0.0059 ± 0.001</td>
<td>52.0 ± 3.0</td>
<td>0.049 ± 0.016</td>
</tr>
<tr>
<td>0.5% CLZ fine particle suspension</td>
<td>0.0045 ± 0.002</td>
<td>155 ± 19</td>
<td>0.014 ± 0.019</td>
</tr>
</tbody>
</table>

The data are presented as means ± S.D. of 5 independent rabbits.

Fig. 5 Changes in IOP of Rabbits following the Instillation of 0.05% CLZ Ophthalmic Solution (A), 0.5% CLZ Fine Particle Suspension (B) or 0.1% Y-27632 Ophthalmic Solution (C).

O: saline; •: solution or suspension containing drugs. The data represent the means ± S.D. of 5 independent rabbits.

Fig. 6 AUC for ΔIOP following the Instillation of 0.05% CLZ Ophthalmic Solution, 0.5% CLZ Fine Particle Suspension or 0.1% Y-27632 Ophthalmic Solution.

The data represent the means ± S.D. of 5 independent rabbits. *p < 0.05 vs. 0.05% CLZ fine particle suspension; **p < 0.05 vs. 0.1% Y-27632 solution.
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400 nm of diameter and 0.027% CLZ for the concentration of soluble CLZ) becomes 12.4%.

The safety of eye drop preparations poses an important problem for the production of useful applications of CLZ ophthalmic solutions and fine particle suspensions. It has been reported that HPβCD has a safety that ranks second to γ-CD among a variety of CD derivatives in used in eye drop formulations. Moreover, Jansen et al. has reported no observable irritation of eye membranes by HPβCD solutions less than 12.5%. The concentrations of HPβCD used in our experiments were approximately one to three concentration (5%) compared with 12.5% in their report. Therefore, the safety of HPβCD concentrations used here appears to be established. Moreover, we used the Draize eye test, a governmentally endorsed method to evaluate the safety of materials meant for use in or around the eyes, and found no stimulation of the eye membrane during the observation period (data not shown), indicating the safety of the HPβCD concentrations used for ophthalmic applications.

CLZ ophthalmic solutions and fine particle suspensions were examined for their ability to reduce the enhanced IOP of rabbits in kept in a darkroom. The instillation of a 0.05% CLZ ophthalmic solution or 0.5% CLZ fine particle suspension to rabbit eyes reduced the enhanced IOP. It has been reported that CLZ is an inhibitor for Type III phosphodiesterase and may inhibit ROCK. Therefore, we also examined the effects of Y-27632, a specific ROCK inhibitor, as a reference drug. In simultaneous experiments using the high-IOP model rabbits, a 0.1% Y-27632 ophthalmic solution (effective time IOP reduction: approximately 240 min) reduced the enhanced IOP longer than the 0.05% CLZ ophthalmic solution (ca. 60 min) or the 0.5% fine particle suspension (ca. 120 min). The differences in the effective times among these drug preparations may arise from differences in their corneal permeabilities and/or specificities to the action site. Therefore, we are now in the progress of investigating changes in the flow of the aqueous humor upon instillation of the CLZ ophthalmic preparations described in this paper. These results obtained to date demonstrate that CLZ ophthalmic solutions and/or fine particle suspension may provide an effective anti-glaucoma formulation.

5 CONCLUSION

The instillation of CLZ ophthalmic solutions and CLZ fine particle suspension effectively reduced the enhanced IOP of rabbits kept in a darkroom. These findings provide significant information that can be used in designing further studies to develop potent anti-glaucoma drugs.

References