Improved Corneal Toxicity and Permeability of Tranilast by the Preparation of Ophthalmic Formulations Containing Its Nanoparticles

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Abstract: We prepared ophthalmic formulations containing 0.5% tranilast (TL) nanoparticles using 0.005% benzalkonium chloride (BAC), 0.5% D-mannitol, and 2-hydroxypropyl- β -cyclodextrin (HP β CD), and investigated their usefulness in the ophthalmologic field by evaluating corneal toxicity and permeability. TL nanoparticles were prepared using zirconia beads and Bead Smash 12, which allowed the preparation of high quality dispersions containing 0.5% TL nanoparticles (particle size, 34 ± 20 nm, means $\pm$ S.D.). Dispersions containing TL nanoparticles are tolerated better by human corneal epithelium cells than a commercially available 0.5% TL preparation (RIZABEN[®] eye drops). In addition, the addition of TL nanoparticles to the dispersions does not affect the antimicrobial activity of BAC against *Escherichia coli* (ATCC 8739), and the corneal penetration of TL from dispersions containing TL nanoparticles was significantly higher than in the case of the commercially available 0.5% TL eye drops. It is possible that dispersions containing TL nanoparticles will show increased effectiveness against ocular inflammation, and that ocular drug delivery systems using drug nanoparticles may lead to an expansion of their usefulness for therapy in the ophthalmologic field.

Key words: nanoparticle, tranilast, eye drops, transcorneal penetration, corneal stimulation

1 INTRODUCTION

The ophthalmic application of drugs is the primary route of administration for the treatment of various eye diseases, and is well-accepted by patients; however, in traditional formulations only small amounts of the administered drug (0.5%) penetrate the cornea to reach the desired intraocular tissue due to corneal barriers and dilution caused by lacrimation¹⁻³⁾. Consequently, there is a need for frequent instillation of concentrated solutions to obtain the desired therapeutic effect in both the anterior and posterior hemispheres of the eye. However, the frequent administration of drugs can cause corneal damage as well as undesirable side effects resulting from the systemic absorption of drugs through the nasolacrimal duct^{4,5)}. Therefore, it is very important to increase the effectiveness of drugs by enhancing their bioavailability⁶⁾. In order to overcome these problems and increase ocular drug bioavailability, several strategies including the preparation of viscous solutions, micro/nanoparticles and hydrogels have been developed and in-

vestigated^{1,4,7-10)}. In the case of viscous solutions, numerous studies have demonstrated that they do not possess sufficient mechanical strength to resist the ocular clearance mechanism, and offer only a transient improvement in ocular residence time¹¹⁾. On the other hand, it has been reported that the penetration capability of drugs across the cornea can be significantly improved by decreasing the particle size using nanoparticles^{3,9,12)}. Implants fabricated using the biodegradable polymer PLGA[poly(DL-lactide-co-glycolide)] with mean particle diameters of 50 – 200 nm have been widely utilized as carriers for bioactive molecules and present a possible solution to the limitations surrounding ocular drug penetration¹³⁻¹⁶⁾. It is expected that ophthalmic drug systems using nanoparticles may provide an alternative strategy for increasing ocular drug penetration¹³⁻¹⁶⁾.

Tranilast (TL), *N*-(3',4'-dimethoxycinnamoyl)anthranilic acid, is a synthetic, multi-potential, anti-allergic, and anti-fibrotic drug reported to have various effects both *in vitro*

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as well as *in vivo*¹⁷). The variety of TL functions published to date include the suppression of collagen synthesis by fibroblasts *via* the down-regulation of cytokine release from monocytes/macrophages¹⁸), the transcriptional and translational inhibition of matrix metallo-proteases in lipopolysaccharide-stimulated neutrophils¹⁹), and the suppression of monocyte/macrophage infiltration and associated myocardial fibrosis in the deoxycorticosterone acetate/salt hypertensive rats²⁰). Because the solubility of TL in water is very low (14.5 µg/ml), it is generally dissolved with the aid of a surface-active agent; in such a solution, TL has been widely used in the ophthalmic field as RIZABEN® eye drops 0.5% solution, and provides effective therapy for ocular inflammation. However, this preparation of TL does not provide effective therapy for uveitis or after cataract surgery because the amount of TL in the intraocular area is low. Therefore, it is possible that enhancing the transcorneal penetration of TL will increase its effectiveness against ocular inflammation (as can occur in uveitis and after cataract surgery), and lead to an expansion of its therapeutic usage in the ophthalmologic field.

Preservatives are essential additives in ophthalmic preparations, and the most common preservative in preparations used to treat glaucoma and ocular surface diseases is BAC, most often at a concentration of 0.01% (range, 0.005 - 0.02%) in topical multi-dose solutions²¹). Although, the BAC is known to cause corneal stimulation, we have previously shown that this side effect can be prevented by the addition of D-mannitol to the preparation²²). In this study, we made preparations of TL nanoparticles containing BAC and D-mannitol, and investigated their usefulness in the ophthalmologic field by evaluating corneal toxicity and permeability.

2 EXPERIMENTAL

2.1 Animals and reagents

Male rabbits were housed under standard conditions (12 h/d fluorescent light (07:00-19:00), 25°C room temperature), and allowed free access to a commercial diet (CR-3, Clea Japan Inc., Tokyo, Japan) and water. All procedures were performed in accordance with the Kinki University Faculty of Pharmacy Committee Guidelines for the Care and Use of Laboratory Animals and the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. TL (TL microparticles) were kindly donated by Kissei Pharmaceutical Co., Ltd. (Nagano, Japan). 2-Hydroxypropyl-β-cyclodextrin (HPβCD, average molar substitution, 0.6; average MW, 1380) was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Commercially available 0.5% TL eye drops (RIZABEN® eye drops 0.5%, TL_{commercially} eye drops) were obtained from Kissei Pharmaceutical Co., Ltd., and benzalkonium chloride

(BAC) was provided by Kanto Chemical Co., Inc. (Tokyo, Japan). Mannitol (D-mannitol) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were of the highest purity commercially available.

2.2 Preparation of ophthalmic dispersions containing TL nanoparticles

TL nanoparticles were prepared using zirconia beads and Bead Smash 12 (a bead mill, Wakenyaku Co. Ltd, Kyoto, Japan). **Scheme 1** shows the preparation method for ophthalmic dispersions containing TL used in this study. Zirconia beads (diameter: 2 mm) were added to TL microparticles (solid, original TL) containing BAC or mannitol, and the mixture was crushed with a Bead Smash 12 for 30 sec (1,500 rpm, 4°C). The mixture was dispersed in saline with or without 5% HPβCD, and crushed with the Bead Smash 12 (5,500 rpm, 60 sec, 4°C) using zirconia beads (diameter: 0.1 mm). The compositions of the dispersions containing TL are shown in **Table 1**. A 0.5% TL dispersion is equivalent to 15.3 mM TL; the pH of both ophthalmic dispersions containing TL micro- or nanoparticles was 6. The particle size was measured using a nanoparticle size analyzer SALD-7100 (Shimadzu Corp., Kyoto, Japan; refractive index 1.60-0.10i).

2.3 Stability of ophthalmic dispersions containing TL

TL concentrations in the samples were determined by a High Performance Liquid Chromatography (HPLC) method. Ten microliters of filtrate was added to 100 µl methanol containing 0.3 µg ethyl *p*-hydroxybenzoate (internal standard), and the mixture was filtered through a Chromatodisk 4A (pore size 0.45 µm, Kurabo Industries Ltd., Osaka, Japan). The solution (10 µl) was injected into an Inertsil® ODS-3 (3 µm, column size: 2.1 mm × 50 mm) column (GL Science Co., Inc., Tokyo, Japan) on a Shimadzu LC-10AD system equipped with a column oven CTO-6A (Shimadzu Corp., Kyoto, Japan). The mobile phase consisted of acetonitrile/50 mM ammonium acetate (20/80) at a flow rate of 0.25 ml/min; the column temperature was 35°C, and the wavelength for detection was 230 nm. Three milliliters of ophthalmic dispersions containing TL as described in **Table 1** were incubated in 5 ml test tubes in the dark at 20°C for 72 h, after which 50 µl of sample solution was withdrawn from 5 mm under the surface at the indicated time intervals (total height of liquid, 4 cm).

2.4 Antimicrobial activity of dispersions containing TL nanoparticles

Dispersions containing TL nanoparticles (TL_{nano}) as described in **Table 1** were tested for antimicrobial activity against *Escherichia coli* (*E. coli*, ATCC 8739). The organism was selected based on Japanese Pharmacopoeia (JP) test protocols, and the minimal inhibitory concentration

Table 1 Ophthalmic Formulations of Particle Dispersions containing TL.

Formulation	Content (w/v%)				Treatment
	TL microparticles	BAC	D-Mannitol	HP β CD	
Milled-TL	0.5	—	—	—	Bead mill
Milled-TL _{BAC}	0.5	0.005	—	—	Bead mill
Milled-TL _{BAC-Mannitol}	0.5	0.005	0.5	—	Bead mill
TL _{micro}	0.5	0.005	0.5	5.0	—
TL _{nano}	0.5	0.005	0.5	5.0	Bead mill

Dispersions containing TL were prepared according to Scheme 1.

Scheme 1 Preparation Procedures of Ophthalmic Dispersions containing TL.

(MIC) was determined from the lowest concentration showing antimicrobial activity²³. According to the standard methodology, the bulk dilution was split into 10 ml aliquots, which were each inoculated with between 10^5 and 10^6 colony-forming units (CFU)/ml of *E. coli* (1 organism per aliquot) and incubated in the presence of vehicle (solution containing 0.005% BAC, 0.5% mannitol and 5% HPβCD) and 0.5% TL-containing dispersions at 20°C to 25°C. The inoculated solutions were sampled and counted on days 2, 7, 14 and 28. One milliliter aliquots were serially diluted in phosphate buffer (pH 7.2), plated in duplicate on soybean-casein digest agar (Casein soya bean digest agar for JP general test, Wako, Osaka, Japan), and incubated at 31°C for 3 days. Raw data counts were converted to log(CFU) values. Since the samples were diluted at least 1:10 at the time of testing, 10 CFU reduction is the lowest sensitivity allowed by the test. In the measurement of MIC, the samples (20 µl) were mixed with 180 µl *E. coli* solution (5×10^4 (CFU)/well) in 96-well microplates (IWAKI, Chiba, Japan), and incubated at 35°C for 24 h.

2.5 Cell culture and treatment

The immortalized human corneal epithelial cell line HCE-T developed by Araki-Sasaki *et al.*²⁴ was used in this study. HCE-T cells were cultured in Dulbecco's modified Eagle's medium/Ham's F12 (GIBCO, Tokyo, Japan) containing 5% (v/v) heat-inactivated fetal bovine serum, 0.1 mg/mL streptomycin and 1000 IU/ml penicillin (GIBCO, Tokyo, Japan). For experiments, HCE-T cells (1×10^4 cells/well) were seeded in 96-well microplates (IWAKI, Chiba, Japan). Ophthalmic dispersions containing 0.5% TL or commercially available 0.5% TL eye drops (200 µl) were added to the cell cultures one day after seeding, and the cells were stimulated for 0 - 120 sec²⁵. Following stimulation, culture medium containing TetraColor One (SEIKAGAKU Co. Tokyo, Japan) was added, the absorbance (Abs) at 490 nm was measured, and cell viability was calculated according to the manufacturer's instructions as represented by equation 1 (Eq. 1):

$$\text{Cell viability (\%)} = \text{Abs}_{\text{stimulation}} / \text{Abs}_{\text{non-stimulation}} \times 100 \quad \text{Eq. 1}$$

where $\text{Abs}_{\text{stimulation}}$ shows the Abs of samples treated with ophthalmic dispersions containing 0.5% TL or commercially available 0.5% TL eye drops. The $\text{Abs}_{\text{non-stimulation}}$ shows the Abs of samples treated with saline.

2.6 In vitro transcorneal penetration of ophthalmic dispersions containing TL

The *in vitro* transcorneal penetration of ophthalmic dispersions containing 0.5% TL or commercially available 0.5% TL eye drops was examined using the method of Iwata *et al.*²⁶ Adult Japanese albino rabbits weighing 2.5 to 3.0 kg 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 on a methacrylate cell designed for transcorneal penetration studies. The side of the chamber (donor chamber) exposed to the exterior surface of the cornea was filled with an ophthalmic dispersion containing 0.5% TL or commercially available 0.5% TL eye drops. The other side of the chamber (reservoir chamber) was filled with 10 mM HEPES buffer (pH 7.4) containing 136.2 mM NaCl, 5.3 mM KCl, 1.0 mM K_2HPO_4 , 1.7 mM CaCl_2 and 5.5 mM glucose. The experiments were performed at 35°C for 6 h. Fifty microliter aliquots of sample solution were withdrawn from the reservoir chamber at the indicated time intervals and replaced with the same volume of buffer. The TL concentrations in the samples were determined by HPLC method described above. Corneal viability was monitored by measuring corneal thickness (0.0625 cm, average for 5 rabbits; no significant changes in thickness were observed over the 6 h period). The obtained data were analyzed according to the following equations:

$$J_c = \frac{K_m \cdot D \cdot C_{\text{TL}}}{\delta} = K_p \cdot C_{\text{TL}} \quad \text{Eq. 2}$$

$$\tau = \frac{\delta^2}{6D} \quad \text{Eq. 3}$$

$$Q_t = J_c \cdot A \cdot (t - \tau) \quad \text{Eq. 4}$$

where J_c is the TL penetration rate, K_m is the cornea/preparation partition coefficient, D is the diffusion constant within the cornea, C_{TL} is the TL content in the ophthalmic preparation, τ is the lag time, δ is thickness of the cornea (0.0625 cm, average of 5 rabbits), Q_t is the total amount of TL appearing in the reservoir solution at time t , and A is the effective area of the cornea (0.78 cm²). J_c and τ were estimated by fitting each penetration profile to Eq. 4. The penetration coefficient through the cornea, K_p , is given by J_c/C_{TL} . A nonlinear least-squares computer program was employed for the calculation²⁵.

2.7 Photochemical resistance of ophthalmic dispersions containing TL

The 0.5% TL_{nano} preparation as described in Table 1 and 0.5% liquid TL in dimethyl sulfoxide were tested for photodegradability under fluorescent light (400 - 700 nm). The TL preparations were exposed to 58 W/m² for 12 h, after which the TL concentrations in 50 µl samples were determined by the HPLC method described above.

2.8 Statistical analysis

All values are presented as mean ± standard deviation (S.D.) or standard error of the mean (S.E.). Unpaired Student's *t*-test was used to evaluate statistical differences, and multiple groups were evaluated by one-way analysis of variance followed by Dunnett's multiple comparison. *P* values less than 0.05 were considered significant.

3 RESULTS

3.1 Preparation of Ophthalmic Dispersions containing TL Nanoparticles

Figure 1 shows the particle size distribution and mean particle diameter of dispersions containing 0.5% TL as described in Table 1. The TL microparticles were milled by the bead mill method to a mean particle size of $8.58 \pm 6.59 \mu\text{m}$ (mean $\pm$ S.D.). Although, the mean particle size of TL was decreased by the addition of BAC, the decrease in particle size was small. On the other hand, TL nanoparticles obtained by the addition of HP β CD had a mean particle size of $34 \pm 20 \text{ nm}$ (mean $\pm$ S.D.). Figure 2 shows the stability of dispersions containing 0.5% TL as described in Table

1. The TL_{micro} preparation precipitated 4 h after preparation. The stability of TL dispersions was increased when the bead mill method was used in conjunction with BAC addition with the Milled-TL_{BAC} preparation precipitating 36 h after preparation. On the other hand, the addition of HP β CD enhanced the stability of the TL dispersion (TL_{nano}), and precipitation was not observed until 72 h after preparation. Figure 3 shows the antimicrobial activities of TL_{nano} preparations. The antimicrobial activity of the 0.005% BAC solution was high with no *E. coli* detected 2 days after treatment. The TL_{nano} preparation also showed high antimicrobial activity approximately equal to that of the 0.005% BAC solution.

Fig. 1 Cumulative Size Distribution and Frequency of 0.5% TL Dispersions with or without BAC, Mannitol and HP β CD. The compositions of the TL dispersions are shown in Table 1. Particle size was determined using a nanoparticle size analyzer SALD-7100 (refractive index 1.60-0.10i). A: cumulative distribution and frequency of TL microparticles (particle size $52.1 \pm 23.4 \mu\text{m}$), B: cumulative distribution and frequency of Milled-TL (particle size $8.58 \pm 6.59 \mu\text{m}$), C: cumulative distribution and frequency of Milled-TL_{BAC} (particle size $8.76 \pm 4.21 \mu\text{m}$), D: cumulative distribution and frequency of Milled-TL_{BAC-Mannitol} (particle size $12.5 \pm 4.10 \mu\text{m}$), E: cumulative distribution and frequency of TL_{nano} (particle size $0.034 \pm 0.020 \mu\text{m}$). The data are presented as means $\pm$ S.D.

Fig. 2 Stability of 0.5% TL Dispersions with or without BAC, Mannitol and HP β CD. 0.5% TL dispersions were kept in the dark at 20°C for 72 h, and the amounts of remaining TL were determined at the indicated time points. The compositions of the TL dispersions are shown in Table 1. The data are presented as means $\pm$ S.E. of 5 independent samples.

Fig. 3 Evaluation of the Antimicrobial Activities of Dispersions with and without TL Nanoparticles. Dispersions containing vehicle alone, vehicle plus 0.005% BAC, or vehicle plus 0.005% BAC and 0.5% TL nanoparticles (TL_{nano}) were tested for antimicrobial activity against *E. coli* (ATCC 8739). Raw data counts were converted to log₁₀ values, and are presented as means $\pm$ S.E. of 5 independent experiments. N.D. indicates not detected. MIC: BAC, 15 μ g/ml; TL_{nano}, 15 μ g/ml.

3.2 Corneal Stimulation and Transcorneal Penetration of Ophthalmic Dispersions containing TL Nanoparticles

Figure 4 shows the changes in the viability of HCE-T cells following treatment with TL_{commercially} eye drops or TL_{micro} or TL_{nano}. The viability of HCE-T cells treated with TL_{commercially} eye drops for 60 or 120 sec was approximately 68.7% or 62.2%, respectively. On the other hand, the via-

Fig. 4 Effects of Dispersions containing 0.5% TL Nanoparticles on the Viability of HCE-T Cells. HCE-T cells in 96-well microplates were treated with commercially available TL eye drops (TL_{commercially} eye drops) or dispersions containing TL microparticles (TL_{micro}) or TL nanoparticles (TL_{nano}) for 0 - 120 sec. Cell viability was calculated using TetraColor One according to Eq. 1 in EXPERIMENTAL. The data are presented as means $\pm$ S.E. of 5-10 experiments. * p < 0.05, vs. TL_{nano}.

bility of HCE-T cells treated with TL_{nano} was higher than in the case of the TL_{commercially} eye drops, and no differences in the viability of HCE-T cells treated with TL_{micro} or TL_{nano} were observed. Figure 5 shows the *in vitro* transcorneal penetration of TL_{commercially} eye drops, TL_{micro}, and TL_{nano} through rabbit corneas, and Table 2 summarizes the pharmacokinetic parameters calculated from the *in vitro* transcorneal penetration data. In the case of TL_{micro}, no TL was detected in the reservoir chamber until 4 h after administration. On the other hand, transcorneal penetration in the case of TL_{commercially} eye drops began after a lag time of 1.93 h with a subsequent penetration rate of 15.2 ± 3.74 nmol/cm²/h (mean $\pm$ S.E.). Penetration from TL_{micro} was lower than that from the commercially available TL eye drops, while the TL penetration rate from TL_{nano} (36.8 ± 1.65 nmol/cm²/h, mean $\pm$ S.E.) was significantly higher in comparison with TL_{commercially} eye drops.

4 DISCUSSION

In this study, we prepared ophthalmic formulations containing TL nanoparticles, and investigated their usefulness in the ophthalmologic field by evaluating their corneal toxicity and permeability.

BAC has been used as an effective preservative and is indispensable in the preparation of eye drops. However, BAC has also been shown to be highly toxic both *in vitro* and *in vivo* due to its stimulatory effect on epithelial cell death^{27,28}.

Fig. 5 *In vitro* Transcorneal Penetration of Dispersions containing TL Nanoparticles. A: Image of the methacrylate cell designed for the transcorneal penetration study. B: Transcorneal penetration of dispersions containing TL nanoparticles. The donor chamber facing the exterior surface of the cornea was filled with commercially available TL eye drops (TL_{commercially} eye drops) or dispersions containing TL microparticles (TL_{micro}) or nanoparticles (TL_{nano}). The data are presented as the means $\pm$ S.E. of 3–4 rabbit corneas. * $p < 0.05$, vs. commercially available TL eye drops (TL_{commercially} eye drops).

BAC is a quaternary ammonium compound that has been shown to hasten the drying of the tear film^{29, 30}, worsen preexisting dry eye³¹, and affect both the cornea and conjunctiva³². In addition, BAC is a pro-inflammatory or pro-apoptotic mediator³³ because it induces oxidative stress^{34–36} or significantly alters precorneal mucins³⁷. Clinically, these iatrogenic effects are found most frequently in eye drops used to treat long-term pathologies and inflammation. The side effects of BAC seem to be both dose- and time-dependent, increasing with larger amounts used for longer periods. On the other hand, we previously reported that the addition of D-mannitol prevents corneal stimulation by

BAC²², and change in chemical structure was not observed by addition of the BAC and D-mannitol²². Therefore, we attempted the preparation of a TL dispersion containing BAC and mannitol using the bead mill method (Milled-TL_{BAC-Mannitol}). Although, the TL particle size in Milled-TL_{BAC-Mannitol} ($12.5 \pm 4.90 \mu\text{m}$) was lower than that of TL microparticles ($52.1 \pm 23.4 \mu\text{m}$, means $\pm$ S.D.), the particle size was not sufficiently small to be called a nanoparticle (Fig. 1), and the stability of Milled-TL_{BAC-Mannitol} was also low (Fig. 2). Mori *et al.*³⁸ reported that adsorption to the surface of cyclodextrin decreases the cohesion of nanoparticulate solids, and we previously reported that the addition of HP β CD is suitable for the preparation of nanoparticles using mill methods³⁹. Furthermore, Jansen *et al.*⁴⁰ have reported no observable irritation of the eye membrane by solutions containing HP β CD at concentrations less than 12.5%. Therefore, we used 5% HP β CD to prepare TL nanoparticle dispersions in this study. The addition of 5% HP β CD resulted in a smaller TL particle size and an increased stability of the resulting dispersions containing TL nanoparticles (TL_{nano}). The antimicrobial activity is important in ophthalmic preparations. TL_{nano} containing 0.005% BAC showed high antimicrobial activity with an MIC equal to that of a 0.005% BAC solution (Fig. 3). These results suggest that the TL nanoparticle dispersions in this study do not affect the antimicrobial activity of BAC, and that the chemical structure shows no difference between TL microparticles and nanoparticles.

Next, we evaluated the dispersions containing TL nanoparticles in terms of corneal damage and transcorneal penetration using HCE-T cells and rabbit corneas (Fig. 4 and 5). The viability of HCE-T cells treated with TL_{nano} was higher than that of cells treated with TL_{commercially} eye drops, and no difference in cell viability was observed between TL_{micro} and TL_{nano} (Fig. 4). In the *in vitro* transcorneal penetration experiment, no TL was detected in the reservoir chamber until 4 h after the introduction of TL_{micro} into the donor chamber (Fig. 5); however, the lag time for the TL_{commercially} eye drops was 1.93 h, and the penetration rate was obviously higher than that from TL_{micro} (Table 2). On the other hand, the penetration from TL_{nano} was significantly higher than that from TL_{commercially} eye drops. Since TL_{nano} contains HP β CD, the data suggest that the higher rate of transcorneal penetration from TL_{nano} is a result of the increased solubility of TL due to its association with HP β CD. Also, the TL penetration rate from dispersions containing 0.5% TL nanoparticles, 0.005% BAC, 0.5% mannitol and 5% HP β CD was approximately 2-fold higher than that from dispersions containing 0.25% TL nanoparticles, 0.005% BAC, 0.5% mannitol and 5% HP β CD (J_c , $15.2 \pm 1.33 \text{ nmol/cm}^2/\text{h}$, means $\pm$ S.E., $n = 3$). These results indicate that the state of the TL nanoparticle itself, that is TL without the formation of the inclusion complex in the presence of HP β CD, is the main factor affecting transcorneal penetra-

Table 2 Pharmacokinetic Parameters for the *in vitro* Transcorneal Penetration of Dispersions containing TL Nanoparticles.

	TL _{micro}	TL _{nano}	TL _{commercially} eye drops
J_c (nmol/cm ² /h)	2.31 ± 0.47	36.8 ± 1.65 ^{*1,2}	15.2 ± 3.74 ^{*1}
k_p (× 10 ⁻³ /h)	0.53 ± 0.31	2.41 ± 0.11 ^{*1,2}	0.99 ± 0.24
k_m	0.41 ± 0.05	0.16 ± 0.01 ^{*1}	0.18 ± 0.03 ^{*1}
τ (h)	2.94 ± 0.33	0.70 ± 0.04 ^{*1,2}	1.93 ± 0.15 ^{*1}
D (× 10 ⁻⁴ cm ² /h)	2.27 ± 0.29	9.37 ± 0.64 ^{*1,2}	3.44 ± 0.30 ^{*1}

Parameters were calculated according to Eqs. 2-4 (see EXPERIMENTAL). TL_{micro}, dispersion containing TL microparticles; TL_{nano}, TL dispersion containing nanoparticles; TL_{commercially} eye drops, commercially available TL eye drops. The data are presented as means ± S.E. of 3-5 independent rabbit corneas. ^{*1} $p < 0.05$, vs. TL_{micro} for each category. ^{*2} $p < 0.05$, vs. commercially available TL eye drops (TL_{commercially} eye drops) for each category.

tion from TL_{nano}. In addition, liquid TL is known to be highly photodegradable, while TL nanoparticles (100 - 200 nm) show high photochemical resistance⁴¹⁾. We also investigated the photochemical resistance of 0.5% TL_{nano} under fluorescent light (400 - 700 nm). Liquid TL in dimethyl sulfoxide was found to be photodegradable when exposed to 58 W/m² for 12 h (remaining content 67.8 ± 4.0% vs. before photo illumination, mean ± S.E. n = 3); however, a high degree of photochemical stability was observed for TL_{nano} (remaining content 98.6 ± 2.7% vs. before photo illumination, mean ± S.E. n = 3). Taken together, TL_{nano} may provide effective therapy for patients with ocular inflammation requiring the long-term application of eye drops (as can occur in uveitis and after cataract surgery). Further studies are needed to elucidate the structure of the delivery system. Therefore, we are now investigating the structure of the delivery system for TL nanoparticles by scanning electron microscopy.

5 CONCLUSIONS

In the present study, we attempted to establish a preparation method for drug nanoparticles, and succeeded in preparing high quality dispersions containing TL nanoparticles (particle size, 34 ± 20 nm, mean ± S.D.). The dispersions containing TL nanoparticles were tolerated better by human corneal epithelium cells than the commercially available 0.5% TL eye drops (RIZABEN[®] eye drops). In addition, the state of the dispersions containing TL nanoparticles did not affect the antimicrobial activity of BAC against *E. coli*, and the corneal penetration of TL from dispersions containing TL nanoparticles was significantly higher than that from commercially available TL eye drops. It is possible that dispersions containing TL nanoparticles will show increased effectiveness in treating ocular inflammation, and an ocular drug delivery system using drug nanoparticles may expand their usage for therapy in the

ophthalmologic field.

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