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Sustained ocular delivery of tilisolol to rabbits after topical administration or intravitreal injection of lipophilic prodrug incorporated in liposomes

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Abstract

To improve the retention time of tilisolol in the precorneal area or vitreous body, we prepared liposomes incorporating the *O*-palmitoyl prodrug of tilisolol. *O*-Palmitoyl tilisolol was completely incorporated in the liposomes. After topical administration of *O*-palmitoyl tilisolol liposomes to the rabbit eye, *O*-palmitoyl tilisolol rapidly disappeared from the tear fluid. The inclusion of 2% carmellose sodium slightly prolonged the retention of *O*-palmitoyl tilisolol in the tear fluid. After intravitreal injection of *O*-palmitoyl tilisolol liposomes, there was a relatively prolonged retention of *O*-palmitoyl tilisolol in the vitreous body. At 24 and 48 h after intravitreal injection of *O*-palmitoyl tilisolol liposomes, the tilisolol concentration in the vitreous body was significantly higher compared with the concentration after intravitreal injection of tilisolol liposomes.

Introduction

Upon topical administration of ophthalmic drugs to the eye, most of the administered amount is rapidly eliminated from the precorneal area due to drainage via the nasolacrimal duct and dilution by tear turnover (Sasaki et al 1999). The cornea, considered a major pathway for ocular penetration of topically applied drugs, is an effective barrier to drug penetration (Sasaki et al 1996, 1997) because the corneal epithelium has annular tight junctions (zonula occludens), which completely surround and effectively seal the superficial epithelial cells. Thus, topical administration on the eye results in low bioavailability due to the short residence time as well as the limited corneal absorption of drugs.

Intravitreal injection has been accomplished clinically by several ophthalmologists to provide more effective therapy through direct delivery of drugs to the site of action in posterior segment disorders (Barza et al 1987; Liu et al 1987; Taniguchi et al 1987, 1988; Peyman & Ganiban 1995). However, they are painful and not convenient for frequent administration. It has been reported that incorporating drugs into liposomes increases half-life and because fewer intravitreal injections are required the toxicity is diminished (Barza et al 1987; Liu et al 1987; Sanborn et al 1992; Hashizoe et al 1994; Peyman & Ganiban 1995). Barza et al (1987)

demonstrated how optimization of liposomes was affected by the lipid composition of the liposome formulation and also showed that after intravitreal injection cholesterol-containing large liposomes were superior to cholesterol non-containing liposomes.

Taking these points into consideration, various ocular drug delivery systems utilizing pharmaceutical modifications have been developed for sustained ocular drug delivery after topical administration (Park & Robinson 1987; Taniguchi et al 1987, 1988; Safwat et al 1994) as well as intravitreal injection (Barza et al 1987; Liu et al 1987; Sanborn et al 1992; Hashizoe et al 1994). An effective approach to optimize the ocular retention of drugs is by the use of liposomes (Barza et al 1987; Liu et al 1987; Taniguchi et al 1987, 1988; Peyman & Ganiban 1995). Liposomes can incorporate essentially any type of drug, either in the polar vehicle interior or in the hydrophobic regions of the lipid bilayers. Hydrophilic drugs would be retained in the aqueous phase of the liposomes (Tanaka et al 1975), whereas moderately lipophilic drugs could be released easily (Sasaki et al 1985; Takino et al 1995). Even if the liposomes exhibited a favourable disposition profile, it must be remembered that rapid release of the incorporated drug would lead to failure to achieve therapeutic potency (Kawakami et al 2000). The physicochemical properties of the drug to be incorporated into the liposomes for ocular drug release must be considered also.

In this study, we have synthesized a lipophilic prodrug of tilisolol, *O*-palmitoyl tilisolol, to control the ocular delivery of tilisolol from liposomes. We evaluated the distribution profiles of *O*-palmitoyl tilisolol incorporated in liposomes after topical administration or intravitreal injection. We have attempted to control the ocular drug retention after topical administration or intravitreal injection using a rational design combining an approach that has included chemical and pharmaceutical modifications. Tilisolol, a β-blocker, was used as the model drug because of its easy detection with fluorescence (Nakagawa et al 1984).

Materials and Methods

Materials

Tilisolol hydrochloride was supplied by Nisshin Flour Milling Co., Ltd (Tokyo, Japan). Palmitoyl chloride was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Egg phosphatidylcholine (egg PC) was kindly supplied by Asahi Chemical Industry Co., Ltd (Tokyo, Japan). All other chemicals were of reagent grade.

Animals

Male Nippon albino rabbits (2.0–3.0 kg) were used throughout the study. The animals were individually housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co., Ltd, Tokyo, Japan). The rabbits were fasted for 24 h before use but had free access to water. The animal experiments conformed to the Guideline for Animal Experimentation in Nagasaki University.

Synthesis of *O*-palmitoyl tilisolol

O-Palmitoyl tilisolol was prepared according to a similar method reported by Sasaki et al (1993).

Preparation of liposomes

Liposome incorporated drugs consisted of egg PC, cholesterol, and drugs at a molar ratio of 3:2:1. The average molecular weight of egg PC was 826. Liposomal lipid concentration was 20 mM. The lipid mixture including drugs was dissolved in chloroform, vacuum-desiccated, and resuspended in 5 mL sterile phosphate-buffered saline (pH 7.4). The suspension was sonicated for 3 min (200 W) at 4°C and the resulting liposomes were extruded five times through 800-μm polycarbonate membrane filters. In addition, liposomes were mixed with equal volumes of 2% carmellose sodium salt solution (carboxymethylcellulose; CMC; Sigma Chemical Co., St Louis, MO).

Evaluation of drugs incorporated in liposomes

The evaluation methods for the incorporation ratio of drugs in liposomes were according to the study of Taniguchi et al (1987). Briefly, the liposome preparations were incubated at 37°C for 30 or 60 min and were ultrafiltrated using a micropartition system (Ultrafree C3, Millipore, Co., Bedford, MA). The drug concentration in the preparation (C_T) and in the ultrafiltrate (C_W) was assayed by HPLC. Thus, the equation for the incorporation ratio in the liposomes was as follows:

$$\text{Incorporation ratio (\%)} = ((C_T - C_W)/C_T) \times 100$$

Topical administration and intravitreal injection experiments

For the topical administration experiment, unanaesthetized rabbits were kept in a prone position on a wooden plate with no restriction of head or eye movement.

Tilisolol or *O*-palmitoyl tilisolol (100 mM) incorporated in liposomes (25 μL) were topically administered onto the cornea of the rabbits. At an appropriate time after topical administration, tear fluid (0.5 μL) was collected with a glass capillary (EM minicaps, Hirschmann Laborgerät, Germany) from the middle of the lower marginal tear strip.

For the intravitreal injection experiment, the rabbits were anaesthetized with an adequate dose of a sodium pentobarbitone solution administered through a marginal ear vein. Tilisolol or *O*-palmitoyl tilisolol (100 mM) incorporated in liposomes (10 μL) was injected directly into the vitreous body. The rabbits were killed by intravenous administration of an overdose of sodium pentobarbitone solution at 4, 24 and 48 h. The vitreous body (0.7–1.0 mL) was collected using a 1.0-mL disposable syringe without needle after the middle of the eyeball was cut with a surgical knife. The samples were subjected to HPLC assay.

Drug assay of tear fluid and the vitreous body

Tear fluid (0.5 μL) and vitreous body (120 μL) samples were mixed with 0.1 M HCl (100 μL) and methanol (100 μL) including the internal standard (300 μg mL^{-1} *o*-ethoxybenzamide for tilisolol, 0.05 μL mL^{-1} benzyl salicylate for *O*-palmitoyl tilisolol). The mixture was centrifuged at 12000 *g* for 10 min and 80 μL supernatant was injected into the HPLC system.

HPLC

An HPLC system (LC-6A, Shimadzu Co., Ltd, Kyoto, Japan) with a fluorescence spectromonitor (RF-530, Shimadzu Co., Ltd, Kyoto, Japan) was used in reverse mode. The excitation wavelength was 315 nm and the emission wavelength was monitored at 420 nm. The stationary phase used was a Cosmosil 5C_{18}-P packed column (150 mm length × 4.6 mm i.d., Nacalai Tesque Inc., Kyoto, Japan). For analysis of tilisolol, the mobile phase was methanol : 50 mM NaH_2PO_4 (39 : 61, v/v) at a flow rate of 1.0 mL min^{-1}. The retention times of tilisolol and *o*-ethoxybenzamide were 4.5 and 11.5 min, respectively. For analysis of *O*-palmitoyl tilisolol, the mobile phase was 2-propanol : acetonitrile : 50 mM NaH_2PO_4 (37 : 33 : 30, v/v) at a flow rate of 1.0 mL min^{-1}. The retention times of *O*-palmitoyl tilisolol and benzyl salicylate were 10.8 and 4.4 min, respectively. The detection limits of tilisolol in the tear fluid and the vitreous body were 7.5 and 0.06 μM, respectively. Similarly, the detection limits of *O*-palmitoyl tilisolol in the tear fluid and the vitreous body were 7.5 and 0.06 μM, respectively.

Results and Discussion

The incorporation ratios of tilisolol and *O*-palmitoyl tilisolol in liposomes at 30 min were 14.8 ± 2.3 % (n = 3) and 100 ± 0 % (n = 3), respectively. The incorporation ratios of tilisolol and *O*-palmitoyl tilisolol between 30 and 60 min were similar (data not shown). It was difficult to prepare completely incorporated tilisolol liposomes because tilisolol was easily released from the liposomes during preparation of the experiments. Therefore, tilisolol liposomes were administered after the tilisolol and liposomes had been incubated for 30 min.

The drug concentration profiles in the tear fluid after topical administration of tilisolol liposomes and *O*-palmitoyl tilisolol liposomes to the eye are shown in Figure 1A. After topical administration of *O*-palmitoyl tilisolol liposomes, *O*-palmitoyl tilisolol rapidly disappeared from the tear fluid and tilisolol was detected at a low concentration. There was little difference in the elimination profiles from the tear fluid between tilisolol after topical administration of tilisolol liposomes and *O*-palmitoyl tilisolol after topical administration of *O*-palmitoyl tilisolol liposomes. *O*-Palmitoyl tilisolol was completely incorporated into the liposomes. Therefore, most of the *O*-palmitoyl tilisolol in the liposomes was probably eliminated in the tear fluid together with the liposomes. This suggested that as well as the incor-

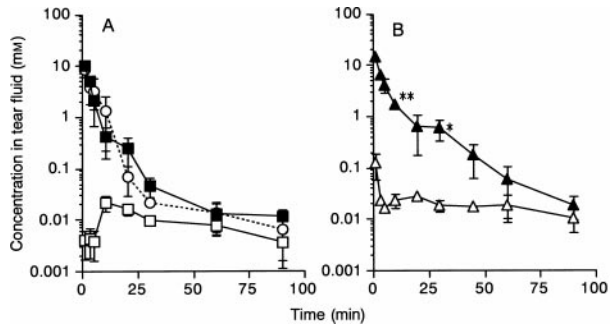

Figure 1 Tear fluid concentration profiles after topical administration of tilisolol liposomes and *O*-palmitoyl tilisolol liposomes (A) and tilisolol liposomes and *O*-palmitoyl tilisolol liposomes with 2% carmellose sodium solution (B) to rabbits. ○, Tilisolol concentration after topical administration of tilisolol liposomes. ■, □, *O*-Palmitoyl tilisolol and tilisolol concentration after topical administration of *O*-palmitoyl tilisolol liposomes, respectively. ▲, △, *O*-Palmitoyl tilisolol and tilisolol concentration after topical administration of *O*-palmitoyl tilisolol liposomes with 2% carmellose sodium solution, respectively. The drug concentration was fixed at 100 mM in all experiments. Each value represents the mean ± s.e. values of at least three experiments. Statistical analysis was performed by analysis of variance. $^*P < 0.05$, $^{**}P < 0.01$ compared with the *O*-palmitoyl tilisolol concentration after topical administration of *O*-palmitoyl tilisolol liposomes.

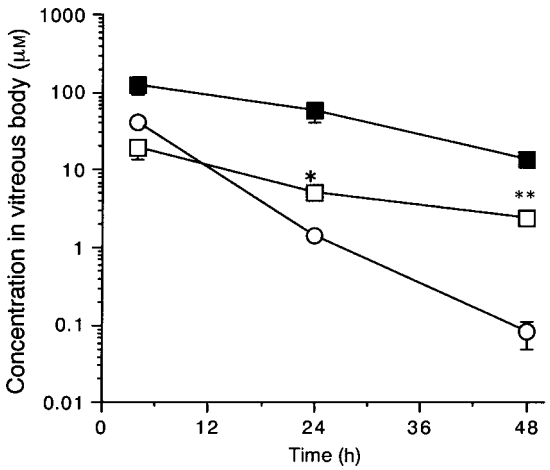

Figure 2 Vitreous body concentration profiles after intravitreal injection of tilisolol liposomes and *O*-palmitoyl tilisolol liposomes to rabbits. ○, Tilisolol concentration after intravitreal injection of tilisolol liposomes. ■, □, *O*-Palmitoyl tilisolol and tilisolol concentration after intravitreal injection of *O*-palmitoyl tilisolol liposomes, respectively. The drug concentration was fixed at 100 mM in all experiments. Each value represents the mean ± s.e. values of at least three experiments. Statistical analysis was performed by analysis of variance. $*P < 0.05$, $**P < 0.01$ compared with the tilisolol concentration after topical administration of tilisolol liposomes.

poration of drug into the liposomes, the elimination of liposomes themselves must be considered for controlling drug delivery after topical administration. Chrai & Robinson (1974) reported that after topical administration using a methylcellulose vehicle in rabbits, the drug concentration in the precorneal area was increased, and the rate of drug elimination decreased following an increase in viscosity. Thus, 2% carmellose sodium was added to the *O*-palmitoyl tilisolol liposomes to retard the liposomal retention in the tear fluid. *O*-Palmitoyl tilisolol concentration at 10 and 30 min after topical administration of 2% carmellose sodium containing *O*-palmitoyl tilisolol liposomes was significantly higher than that of *O*-palmitoyl tilisolol liposomes (Figure 1B). The retention of carmellose sodium in the tear fluid may act as a reservoir for liposomes.

After intravitreal injection of *O*-palmitoyl tilisolol liposomes, *O*-palmitoyl tilisolol was retarded in the vitreous body compared with tilisolol after injection of tilisolol liposomes (Figure 2). After intravitreal injection of *O*-palmitoyl tilisolol liposomes the tilisolol concentration at 24 and 48 h was significantly higher compared with the concentration of tilisolol after injection of tilisolol liposomes. *O*-Palmitoyl tilisolol formulated the micellar solution because of its amphiphilic properties (Kawakami et al 2001). Previously, we reported that the *O*-palmitoyl tilisolol micellar solution enhanced retention in the tear fluid after topical administration. However, the volume of vitreous body (approximately 1.2 mL) (Ohtori & Tojo 1994) was much higher than that of tear fluid (approximately 7.5 μL) (Chrai et al 1973). Thus, after intravitreal injection the micellar solution was diluted by the solution in the vitreous body and aggregation was induced under their critical micelle concentration (Cheng et al 1999). Therefore, from the viewpoint of toxicology, intravitreal injection of *O*-palmitoyl tilisolol micellar solution was limited.

In conclusion, *O*-palmitoyl tilisolol was eliminated from the tear fluid together with liposomes after topical administration of *O*-palmitoyl tilisolol liposomes. Further pharmaceutical modifications such as the use of carmellose sodium appeared to control drug concentration in the precorneal area. In contrast, *O*-palmitoyl tilisolol incorporated in liposomes enhanced the retention of *O*-palmitoyl tilisolol in the vitreous body after intravitreal injection.

References

Barza, M., Stuart, M., Szoka, F. (1987) Effect of size and lipid composition on the pharmacokinetics of intravitreal liposomes. *Invest. Ophthalmol. Vis. Sci.* **28**: 893–900

Cheng, L., Hostetler, K. Y., Gardner, M. F., Avila, C. P., Bergeron-Lynn, G., Keefe, K. S., Wiley, C. A., Freeman, W. R. (1999) Intravitreal toxicology in rabbits of two preparations of 1-*O*-octadecyl-*sn*-glycerol-3-phosphonoformate, a sustained-delivery anti-CMV drug. *Invest. Ophthalmol. Vis. Sci.* **40**: 1487–1495

Chrai, S. S., Patton, T. F., Mehta, A., Robinson, J. R. (1973) Lacrimal and instilled fluid dynamics in rabbit eyes. *J. Pharm. Sci.* **62**: 1112–1121

Chrai, S. S., Robinson, J. R. (1974) Ocular evaluation of methylcellulose vehicle in albino rabbits. *J. Pharm. Sci.* **63**: 1218–1223

Hashizoe, M., Ogura, Y., Kimura, H., Moritera, T., Honda, Y., Kyo, M., Hyon, S.-H., Ikada, Y. (1994) Scleral plug of biodegradable polymers for controlled drug release in the vitreous. *Arch. Ophthalmol.* **112**: 1380–1384

Kawakami, S., Munakata, C., Fumoto, S., Yamashita, F., Hashida, M. (2000) Targeted delivery of prostaglandin E_1 to hepatocytes using galactosylated liposomes. *J. Drug Target.* **8**: 137–142

Kawakami, S., Nishida, K., Mukai, T., Yamamura, K., Sakaeda, T., Nakamura, J., Nakashima, M., Sasaki, H. (2001) Ocular absorption behavior of palmitoyl tilisolol, an amphiphilic prodrug of tilisolol, for ocular drug delivery. *J. Pharm. Sci.* In press

Liu, K.-R., Peyman, G. A., Khoobehi, B., Alkan, H., Fiscella, R. (1987) Intravitreal liposome-encapsulated trifluorothymidine in a rabbit model. *Ophthalmology* **94**: 1155–1159

Nakagawa, Y., Sugai, T., Chin, W. P., Shibuya, T., Hashimoto, K., Imai, S. (1984) Pharmacological profile of a new β-adrenoceptor blocker, 4-[3-(*tert*-butylamino)-2-hydroxypropoxy]-*N*-methylisocarbostyril hydrochloride (N-696). *Arzneimittelforschung* **34**: 194–199

Ohtori, A., Tojo, K. (1994) In vivo/in vitro correlation of intravitreal

delivery of drugs with the help of computer simulation. *Biol. Pharm. Bull.* **17**: 283–290

Park, H., Robinson, J. R. (1987) Mechanisms of mucoadhesion of poly(acrylic acid) hydrogels. *Pharm. Res.* **4**: 457–464

Peyman, G. A., Ganiban, G. J. (1995) Delivery systems for intraocular routes. *Adv. Drug Del. Rev.* **16**: 107–123

Safwat, S. M., Kassem, M. A., Attia, M. A., El-Mahdy, M. (1994) The formulation performance relationship of multiple emulsions and ocular activity. *J. Control. Rel* **32**: 259–268

Sanborn, G. E., Anand, R., Torti, R. E., Nightingale, S. D., Cal, S. X., Yates, B., Ashton, P., Smith, T. (1992) Sustained-release ganciclovir therapy for treatment of cytomegalovirus retinitis: use of an intravitreal device. *Arch. Ophthalmol.* **110**: 188–195

Sasaki, H., Kakutani, T., Hashida, M., Sezaki, H. (1985) Absorption characteristics of the lipophilic prodrug of mitomycin C from injected liposomes or an emulsion. *J. Pharm. Pharmacol.* **37**: 461–465

Sasaki, H., Igarashi, Y., Nishida, K., Nakamura, J. (1993) Ocular delivery of the β-blocker, tilisolol, through the prodrug approach. *Int. J. Pharm.* **93**: 49–60

Sasaki, H., Ichikawa, M., Kawakami, S., Yamamura, K., Nishida, K., Nakamura, J. (1996) In situ ocular absorption of tilisolol through ocular membranes in albino rabbits. *J. Pharm. Sci.* **85**: 940–943

Sasaki, H., Ichikawa, M., Kawakami, S., Yamamura, K., Mukai, T., Nishida, K., Nakamura, J. (1997) In-situ ocular absorption of ophthalmic β-blockers through ocular membranes in albino rabbits. *J. Pharm. Pharmacol.* **49**: 140–144

Sasaki, H., Yamamura, K., Mukai, T., Nishida, K., Nakamura, J., Nakashima, M., Ichikawa, M. (1999) Enhancement of ocular drug penetration. *Crit. Rev. Ther. Drug Carrier Syst.* **16**: 85–146

Takino, T., Nagahama, E., Sakaeda, T., Yamashita, F., Takakura, Y., Hashida, M. (1995) Pharmacokinetic disposition analysis of lipophilic drugs injected with various lipid carriers in the single-pass rat liver perfusion system. *Int. J. Pharm.* **114**: 43–54

Tanaka, T., Taneda, K., Kobayashi, H., Okumura, K., Muranishi, S., Sezaki, H. (1975) Application of liposomes to the pharmaceutical modification of the distribution characteristics of drugs in the rat. *Chem. Pharm. Bull.* **23**: 3069–3074

Taniguchi, K., Yamazawa, N., Itakura, K., Morisaki, K., Hayashi, S. (1987) Partition characteristics and retention of anti-inflammatory steroids in liposomal ophthalmic preparations. *Chem. Pharm. Bull.* **35**: 1214–1222

Taniguchi, K., Itakura, K., Yamazawa, N., Morisaki, K., Hayashi, S., Yamada, Y. (1988) Efficacy of a liposome preparation of anti-inflammatory steroid as an ocular drug-delivery system. *J. Pharmacobio-Dyn.* **11**: 39–46