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Co-instillation of nano-solid magnesium hydroxide enhances corneal permeability of dissolved timolol

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ABSTRACT

We prepared magnesium hydroxide (MH) nanoparticles by a bead mill method, and investigated whether the co-instillation of MH nanoparticles improves the low transcorneal penetration of water-soluble drugs, such as the anti-glaucoma eye drug timolol maleate (TM). MH particle size was decreased by the bead mill treatment to a mean particle size of 71 nm. In addition, the MH nanoparticles were highly stable. Next, we demonstrated the effect of MH nanoparticles on the corneal surface. MH shows only slight solubility in lacrimal fluid, and the instillation of MH nanoparticles for 14 days did not affect the behavior (balance of secretion and excretion) of the lacrimal fluid in rabbit corneas. Moreover, there was no observable corneal toxicity of MH nanoparticles, and treatment with MH nanoparticles enhanced the intercellular space ratio in the eyes of rats. MH alone did not permeate into the cornea; however, the co-instillation of MH nanoparticles and dissolved TM (nMTFC) enhanced the corneal penetration of TM. In addition, the intraocular pressure (IOP)-reducing effect of nMTFC was significantly higher than those of the TM solution or the co-instillation of MH microparticles and TM. In conclusion, we found that MH nanoparticles enhance the corneal penetration of dissolved TM with no observable corneal stimulation or obstruction of the nasolacrimal duct by the MH nanoparticles. It is possible that the co-instillation of MH nanoparticles may provide a useful way to improve the bioavailability of water-soluble drugs in the ophthalmic field. These findings provide significant information that can be used to design further studies aimed at developing anti-glaucoma eye drugs.

1. Introduction

Glaucoma, which involves a long-term elevation in intraocular pressure (IOP), can lead to damage of the optic nerve, resulting in vision impairment and even blindness. Reducing IOP remains the only treatment proved to effectively prevent the development and progression of glaucoma. Therefore, decreasing and maintaining IOP is the most direct method for treating glaucoma, and medical therapy using topical eye drops is the first line and preferred method for treating glaucoma.

Ophthalmic formulation (eye drops) conteining timolol maleate (TM), in anti-glaucoma eye drops, is a nonselective β-blocker. The mechanism of action of TM probably involves a reduction in the formation of aqueous humor in the ciliary body of the eye (Grieshaber and Flammer, 2010). Several combinations of other glaucoma drugs and TM have been introduced (European Glaucoma Society, 2016) because TM has a different mechanism of action as compared to prostaglandin analogs, carbonic anhydrase inhibitors, and miotics. On the other hand, TM is water-soluble, and its topical application TM eye drops results in low bioavailability through the hydrophobic cornea epithelium. In addition, the

Abbreviations: Abs, absorbance; *AUC*, area under the timolol concentration-time curve; BAC, benzalkonium chloride; DDS, drug delivery systems; Eq, equation; MH, magnesium hydroxide; IOP, intraocular pressure; MC, methylcellulose; $MgCl_2$, magnesium chloride; mMH, MH microparticle; *MRT*, mean residence time; MTFC, MH/TM fixed combination; MW, magnesium water; nMH, MH nanoparticle; TM, timolol maleate.

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remaining drug that enters the systemic circulation can cause side effects, such as bronchial asthma. For these reasons, improvement of the low bioavailability of TM is highly anticipated.

In general, simple drug delivery systems (DDS) for glaucoma therapy enhanced the cornealpenetration of drugs, and implants (Aburahma and Mahmoud, 2011; Deokule et al., 2012; Mealy et al., 2014), hydrogels (Cho et al., 2016), microspheres (Fedorchak et al., 2014; Chiang et al., 2016), microparticles and nanoparticles have been investigated as DDS for the effective management of glaucoma. We have found that ophthalmic formulations containing nanoparticles enhance the corneal penetration drugs containing disulfiram, indomethacin and cilostazol (Nagai et al., 2014, 2015a, 2015b). Changes on the corneal surface when nanoparticles are instilled may also affect the corneal penetration of water-soluble drugs, and thus may allow the development of novel DDS.

The selection of the material for the design of combination eye drops is important. Magnesium hydroxide (MH, brucite) is an alkaline earth metal hydroxide that can be found in nature. MH is insoluble in water (solubility in water 0.009% w/w, 18 °C). MH has a wide range of applications including in the removal of contaminants from water (Cao et al., 2012; Li et al., 2015; Wang et al., 2016), as an antibacterial (Dong et al., 2010, 2014; Pan et al., 2013), in gas adsorption (Du et al., 2012), and as a catalyist in the hydrogen evolution reaction (Dai et al., 2016). In addition, MH and its additives are used in medicines, such as antacids and laxatives. In this study, we designed MH nanoparticles (nMH) by the bead mill method, and investigated the changes in the cornea after the instillation of nMH. In addition, we looked at whether the co-instillation of nMH and dissolved TM enhances the bioavailability and IOP-reducing effect of TM.

2. Materials and methods

2.1. Reagents and animals

TM and 0.4% Benoxil were purchased from Sigma-Aldrich Co. LLC (Osaka Japan) and Santen Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. Magnesium hydroxide (MH), magnesium chloride ($MgCl_2$), methylcellulose SM-4 (MC), mannitol and the Magnesium B test kit were provided by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Benzalkonium chloride (BAC) was obtained from by Kanto Chemical Co., Inc. (Tokyo, Japan). Fluorescein was provided by Alcon Japan (Tokyo, Japan). Cell Count Reagent SF was purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals were used the highest purity commercially available. Rabbits (Japanese albino, 2.5 kg) and rats (Wistar, 7 weeks) were provided by Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan) and Kiwa Laboratory Animals Co., Ltd. (Wakayama, Japan), respectively, and housed under standard conditions. All experiments were performed in accordance with the Kindai University Faculty of Pharmacy Committee Guidelines for the Care of Laboratory Animals and the ARVO resolution on the use of animals in research.

2.2. Preparation of MTFC ophthalmic formulation

TM was dissolved in saline with BAC and mannitol (TM solution). nMH in saline with BAC and mannitol was prepared using a bead mill method according to our previous study (Nagai et al., 2015b). The MH/TM fixed combination (MTFC) was prepared by mixing the TM solution and MH particles. Table 1 shows the compositions of the ophthalmic formulations containing MH and/or TM dispersions. During preparation, the solvent containing additive was filtered through a Minisart CE 0.20 μm (Costar, Cambridge, MA, USA) in an aseptic technique. Magnesium water (MW) was prepared from $MgCl_2$. The eye drop preparations were subjected to isotonization, and the pH levels were adjusted to 8.5. A nano particle size analyzer (SALD-7100, Shimadzu Corp., Kyoto, Japan, refractive index 1.60−0.10i) and scanning probe microscope (SPM-9700, Shimadzu Corp., Kyoto, Japan) were used to measure the particle-size and to obtain a particle image. The TM concentration was determined using a Shimadzu LC-20AT system (Shimadzu Corp., Kyoto, Japan) at 294 nm (HPLC method) using 25 mM phosphate buffer/methanol/acetonitrile (60/30/10, v/v, pH 7) as the mobile phase. Other conditions were as follows: internal standard, 10 μg propyl p-hydroxybenzoate; column, Mightysil RP-18 (3 μm, Kanto Chemical Co., Inc., Tokyo, Japan); column oven, 35 °C; flow rate of mobile phase, 0.2 ml/min.

Table 1
Compositions of ophthalmic formulations containing MH and/or TM.

Formulation	MH	TM	MC	BAC	Mannitol	$MgCl_2$	Treatment
MW	–	–	0.5%	0.005%	0.5%	0.01%	–
TM solution	–	0.5%	0.5%	0.005%	0.5%	–	–
mMH	0.01%	–	0.5%	0.005%	0.5%	–	–
nMH	0.01%	–	0.5%	0.005%	0.5%	–	Bead mill
mMTFC	0.01%	0.5%	0.5%	0.005%	0.5%	–	–
nMTFC	0.01%	0.5%	0.5%	0.005%	0.5%	–	Bead mill

MW, magnesium water. mMH, dispersions containing magnesium hydroxide microparticles. nMH, dispersions containing magnesium hydroxide nanoparticles. mMTFC, mMH/TM fixed combination. nMTFC, nMH/TM fixed combination.

2.3. Stability of ophthalmic formulations containing MH

The dispersions containing MH microparticles (mMH) or nMH was preserved in the dark for 8 days at 20 °C, and a sample of 8−10% from the upper part of the solution was withdrawn. The MH was dissolved in HCl, and measured by using a Magnesium B test kit according to the manufacturer's instructions.

2.4. Evaluation of in vitro corneal toxicity of MH using HCE-T cells

The immortalized human corneal epithelial cell line HCE-T (1×10^4 cells) was stimulated with 0.01% MH for 0−120 s in 96-well microplates (IWAKI, Chiba, Japan). Then, Cell Count Reagent SF was added, and the absorbance (Abs) at 490 nm was measured. Cell viability was calculated according to the manufacturer's instructions as represented by Eq. (1):

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

2.5. Evaluation of in vivo toxicity of MH using rabbit eye

Thirty microliters of 0.01% MH were instilled into the eyes of rabbits twice a day (9:00 and 19:00) for 14 days. The amount of lacrimal fluid was measured by the Schirmer's test using Sterilized Tear Production Measuring Strips (SHOWA YAKUHIN KAKO Co., Ltd., Tokyo, Japan). The wound area was stained with 1% fluorescein and measured using a TRC-50X fundus camera (Topcon, Tokyo, Japan) equipped with a digital camera.

2.6. Measurement of in vivo corneal toxicity of MH using rat eyes

The experiment was performedusing rat debrided corneal epitheliumRats were anesthetized with isoflurane and 0.4% Benoxil, and the corneal epithelium was debrided. The debrided areas were as follows: none, 11.95 ± 0.43 mm^2; vehicle, 12.06 ± 0.45 mm^2;