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A nanoparticle formulation of disulfiram prolongs corneal residence time of the drug and reduces intraocular pressure

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ABSTRACT

The goal in the search for successful therapies for glaucoma is the reduction of intraocular pressure (IOP), and the search for effective eye drops that reduce IOP is a high priority. We previously reported the potential of a 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution containing 0.5% DSF (DSF solution) to provide effective anti-glaucoma treatment in eye drop form. In this study, we designed new ophthalmic formulations containing 0.5% DSF nanoparticles prepared by a bead mill method (DSF_{nano} dispersion; particle size 183 ± 92 nm, mean ± S.D.), and compared the IOP-reducing effects of a DSF_{nano} dispersion with those of a DSF solution. The high stability of the DSF_{nano} dispersion was observed until 7 days after preparation, and the DSF_{nano} dispersion showed high antimicrobial activity against *Escherichia coli* (ATCC 8739). In transcorneal penetration experiments using rabbit corneas, only diethyldithiocarbamate (DDC) was detected in the aqueous humor, while no DSF was detected. The DDC penetration level (area under the curve, *AUC*) and corneal residence time (mean residence time, *MRT*) of the DSF_{nano} dispersion were approximately 1.45- and 1.44-fold higher than those of the DSF, respectively. Moreover, the IOP-reducing effects of the DSF_{nano} dispersion were significantly greater than those of the DSF solution in rabbits (the IOP was enhanced by placing the rabbits in a dark room for 5 h). In addition, DSF_{nano} dispersion are tolerated better by a corneal epithelial cell than DSF solution and commercially available timolol maleate eye drops. It is possible that dispersions containing DSF nanoparticles will provide new possibilities for the effective treatment of glaucoma, and that an ocular drug delivery system using drug nanoparticles may expand their usage as therapy in the ophthalmologic field. These findings provide significant information that can be used to design further studies aimed at developing anti-glaucoma drugs.

1. Introduction

Glaucoma is one of the most common causes of visual impairment and blindness in the world, and is more common in the elderly (Quigley, 1996). The major risk factor for glaucoma is enhanced intraocular pressure (IOP). If the IOP is high or is remains at an elevated level for a long period of time, apart from the damage to the ciliary artery, there is mechanical damage to the optic nerve in the central visual areas (Guo et al., 2005). In treatments for glaucoma, the focus is on reducing IOP, thus limiting damage to the retina and optic nerve. The principal pharmacological agents include β-blockers, prostaglandin agents, topical carbonic anhydrase inhibitors, α_1-blockers, α_2-agonists, α,β-blockers and parasympathomimetic agents, all which focus on reducing elevated IOP, since the retinal and optic nerve damage that result from elevated IOP are not satisfactorily controlled by the current therapies. Thus, the goal in the search for successful therapies for glaucoma is the reduction of IOP, and the search for effective eye drops that reduce IOP is a high priority.

Disulfiram (DSF), a dimer of diethyldithiocarbamate (DDC), has long been used to treat alcoholic syndrome without severe side effects (Ito et al., 1999). DSF is a potent nitric oxide synthase (NOS) inhibitor and radical scavenger (Ito et al., 1999; Nabekura et al.,

Abbreviations: AUC, area under the curve; *AUMC*, area under the first moment curve; BAC, benzalkonium chloride; CFU, colony-forming units; DDC, diethyldithiocarbamate; DSF, disulfiram; Eq, equation; HPβCD, 2-hydroxypropyl-β-cyclodextrin; IOP, intraocular pressure; JP, japanese pharmacopoeia; Mannitol, D-mannitol; MC, methylcellulose; *MRT*, mean residence time; NO, nitric oxide; NOS, nitric oxide synthase; S.D., standard deviation; S.E, standard error.

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2003; Nagai and Ito, 2007; Nagai et al., 2008b, 2008c; 2014d; Kanai et al, 2010). However, its application in the ophthalmic field is limited due to its poor water solubility. One problem with lipophilic drugs such as DSF administered as aqueous eye drops lies in obtaining the desired drug concentration in the drug delivery system. In a previous study, we prepared a 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution containing DSF and low-substituted methylcellulose (MC, METOLOSE SM-4) (DSF solution) (Ito et al., 2010; Nagai et al., 2012, 2014c). It is possible that the ability of DSF solution to prevent nitric oxide (NO) production may provide a new anti-glaucoma treatment, and so it is very important to increase the efficacy of DSF solution.

On the other hand, it has recently been reported that the drug penetration capability across the cornea can be significantly improved by decreasing the particle size of the drug using nanoparticles (Rafie et al., 2010; Gupta et al., 2011; Li et al., 2012). It is expected that ophthalmic drug systems using nanoparticles will improve the problem of poor water solubility in eye drops preparations of lipophilic drugs, and provide an alternative strategy for increasing ocular drug penetration (Cohen et al., 1991; Tomoda et al., 2011, 2012).

There are two major approaches to the design of nanocarriers: bottom-up synthesis and a top-down approach (Bacher et al., 1998; Desai et al., 1999; Bender et al., 2000; Matthew et al., 2001; Resnick et al., 2005; Fang et al., 2006; Caldorera-Moore et al., 2010; Dasgupta et al., 2013; Probst et al., 2013). Bottom-up synthesis, which is based on self assembly and emulsion systems, has been studied extensively in the past, and a variety of potential nanocarriers have been developed using this method, for example, polymeric nanoparticles, micelles, liposomes, nanoemulsions, dendrimers, biodegradable and non-biodegradable carriers, solid lipid nanoparticles, magnetic nanoparticles etc. A majority of these carriers are colloidal systems governed by varying forces such as hydrophobic interactions, Van der Waals forces, hydrogen bonding, and ionic interactions. However, major advancements have recently been made in fabrication technology by the introduction of the "top-down" approach for micro and nano-fabrication systems using electromechanical techniques. This approach shows the potential to design nanoparticles with high precision in terms of particle shape and size. We have also designed ophthalmic formulations containing drug nanoparticles obtained by mill methods (Nagai and Ito, 2014a, 2014b, 2014c; Nagai et al., 2014a, 2014b), which can provide high quantity dispersions containing drug nanoparticles by a simple operation. Our previous report showed that dispersions containing tranilast and indomethacin nanoparticles prepared by a bead mill method showed enhanced corneal penetration as compared with commercially available eye drops (drug solutions) (Nagai and Ito, 2014a; Nagai et al., 2014a, 2014b). It is possible that enhancing the transcorneal penetration of DSF will increase its effectiveness against glaucoma, and lead to an expansion of its usage for therapy in the ophthalmologic field.

In this study, we attempted to prepare ophthalmic dispersions containing DSF nanoparticles (DSF_{nano} dispersion) using zirconia beads and Bead Smash 12 (a bead mill, Wakenyaku Co. Ltd, Kyoto, Japan), and investigated the corneal permeability of these ophthalmic formulations containing DSF nanoparticles. In addition, we demonstrated the effect of these ophthalmic formulations on IOP using rabbits kept in the dark.

2. Materials and methods

2.1. Animals and materials

Male Japanese albino rabbits, 2.5–3.0 kg, were housed under standard conditions (12 h/day fluorescent light (07:00–19:00), 25 °C room temperature), 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 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. DSF (solid, original DSF, mean particle size 97.4 ± 36.9 μm, means ± S.D.) was kindly donated by Ouchi Shinko Chemical Industrial Co., Ltd. (Tokyo, Japan). 2-Hydroxypropyl-β-cyclodextrin (HPβCD, average molar substitution, 0.6; average MW, 1380) was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Low-substituted methylcellulose (MC, METOLOSE SM-4, average viscosity, 4 Pa s at 20 °C) was provided by Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Benzalkonium chloride (BAC) was obtained from 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 an ophthalmic solution containing DSF along with HPβCD

HPβCD was added to saline containing 0.001% BAC along with DSF microparticles (solid, original DSF). The mixture was stirred for

Table 1
Ophthalmic formulations, pharmacokinetic parameters for the transcorneal penetration and IOP-reducing effect of solution and dispersion containing DSF.

Formulation	Content (w/v%)					Treatment
	DSF microparticles	BAC	D-mannitol	HPβCD	MC	
DSF solution	0.5	0.001	0.1	5.0	0.5	–
DSF_{nano} dispersion	0.5	0.001	0.1	–	0.5	Bead mill

In vitro transcorneal penetration	J_c (nmol cm^{-2}·min^{-1})	k_p (× 10^{-4}·min^{-1})	k_m (× 10^{-2})	τ (min)	D (× 10^{-5} cm^2·min^{-1})
DSF solution	1.61 ± 0.05	5.76 ± 0.06	5.3 ± 0.2	0.98 ± 0.42	66.2 ± 20.0
DSF_{nano} dispersion	1.59 ± 0.05	5.75 ± 0.04	46.1 ± 0.8*	8.71 ± 2.90*	7.47 ± 1.9*

In vivo transcorneal penetration	k_a (× 10^{-2}·min^{-1})	F (%)	τ (min)	AUC_{DDC} (μM·min)	MRT_{DDC} (min)
DSF solution	8.23 ± 0.71	1.86 ± 0.19	3.99 ± 0.09	3438 ± 127	36.3 ± 2.7
DSF_{nano} dispersion	1.12 ± 0.16*	7.37 ± 0.65*	7.17 ± 0.21*	4981 ± 149*	52.2 ± 3.4*

IOP-reducing effect	ΔAUC_{IOP} (mmHg·min)	ΔMRT_{IOP} (min)
DSF solution	576.5 ± 48.6	82.5 ± 4.5
DSF_{nano} dispersion	929.7 ± 61.2*	151.4 ± 6.3*

Parameters were calculated according to Eqs. (1)–(3), (5)–(11) (see Materials and methods). The data are presented as means ± S.E. of 7 independent rabbit corneas. *$P < 0.05$, vs. DSF solution for each category.