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Topical drug delivery to retinal pigment epithelium with microfluidizer produced small liposomes

T. Lajunen [a,b], K. Hisazumi [c], T. Kanazawa [a], H. Okada [a], Y. Seta [a], M. Yliperttula [b], A. Urtti [b,d], Y. Takashima [a,*]

[a] Tokyo University of Pharmacy & Life Sciences, Japan
[b] Centre for Drug Research, Division of Pharmaceutical Biosciences, University of Helsinki, Finland
[c] Powrex Corporation, Itami, Japan
[d] School of Pharmacy, University of Eastern Finland, Finland

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ABSTRACT

Drug delivery from topically instilled eye drops to the posterior segment of the eye has long been one of the greatest challenges of ocular drug development. We developed methods of liposome preparation utilizing a microfluidizer to achieve adjustable nanoparticle size (even less than 80 nm) and high loading capacity of plasmid DNA. The microfluidizing process parameters were shown to affect the size of the liposomes. Higher operating pressures and passage for at least 10 times through the microfluidizer produced small liposomes with narrow size distribution. The liposomes were physically stable for several months at +4 °C. *In vivo* distribution of the optimized liposome formulations in the rat eyes was investigated with confocal microscopy of the histological specimens. Transferrin was used as a targeting ligand directed to retinal pigment epithelium. Size dependent distribution of liposomes to different posterior segment tissues was seen. Liposomes with the diameter less than 80 nm permeated to the retinal pigment epithelium whereas liposomes with the diameter of 100 nm or more were distributed to the choroidal endothelium. Active targeting was shown to be necessary for liposome retention to the target tissue. In conclusion, these microfluidizer produced small liposomes in eye drops are an attractive option for drug delivery to the posterior segment tissues of the eye.

* Corresponding author. Address: 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan. Tel./fax: +81 42 676 4492.
E-mail address: takasima@toyaku.ac.jp (Y. Takashima).

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1. Introduction

The posterior segment of the eye remains as a difficult target for drug delivery (Urtti, 2006). Only the anterior part of the eye can be treated with relative ease by using topical instilled eye drops. So far, the most common delivery approach to the ocular posterior segment has been the direct injections into the vitreal cavity (Del Amo and Urtti, 2008). Intravitreal injections must be applied by medical professionals, frequent dosing is required and such drug administrations are burdensome to the patients and health care system. For example, exudative form of age-related macular degeneration (AMD) is treated monthly by bilateral intravitreal antibody injections for the rest of the patients' lives. Because the use of repeated invasive injections involves a risk of infections and other ocular complications, broad spectrum antibiotic treatment follows every injection in large patient populations, a procedure that might give rise to evolution of resistant bacterial strains. The monthly dosing regimen is feasible with the antibodies (Prager et al., 2009) that usually have half-life about one week in the vitreous (Bakri et al., 2007; Kim et al., 2006; Mordenti et al., 1999), but still the convenience and the non-dependence on medical professionals of topical drug delivery cannot be achieved. Furthermore this approach is not suitable for small molecular weight drugs with typical half-lives of 1–10 h in the vitreous (Kidron et al., 2012). For small molecules, slowly dissolving suspensions or sophisticated controlled release implants can be used (Nagai et al., 2014), but the invasive nature and many associated problems of drug delivery are not avoided. Therefore, the controlled release implants are not used widely in the clinics. Non-invasive drug delivery methods, amenable to out-patient use, would constitute major improvement in the management of the retinal diseases, such as age related macular degeneration, diabetic retinopathy, macular edema and retinal apoptosis in glaucoma.

At the ocular surface eye drops are rapidly cleared and corneal epithelium hinders drug absorption into the inner eye (Maurice and Mishima, 1984; Urtti, 2006). The classical trans-corneal route of drug entry into the eye is only suitable for lipophilic small molecules, because it consists of one lipophilic layer with tightly packed cells and two hydrophilic layers (Beuerman and Pedroza, 1996; Huang et al., 1983). Compared to the cornea the permeability of conjunctiva is higher and more amenable also to large molecules (Horibe et al., 1997; Hämäläinen et al., 1997). Also, non-corneal route is more favorable in the delivery of macromolecules than small molecules, because their loss to the systemic blood circulation is less (Ranta et al., 2010). Nevertheless, the drug delivery via the non-corneal route into the posterior eye segment is a complicated process that involves tissue barriers (conjunctiva, sclera, retinal pigment epithelium) and fluid flow factors, such as conjunctival and choroidal elimination to the blood flow (Ranta et al., 2010). This reduces the posterior segment bioavailability small molecular weight drugs, like timolol, to the range of 0.01% (Maurice, 2002; Urtti et al., 1990). Conjunctival capillaries are fenestrated and transfer from epithelium to the blood vessels has few barriers for macromolecules and nanoparticles (Krachmer et al., 2011). Blood circulation distributes the drug around the eye, thereby helping the delivery process. Conjunctival artery and ciliary arteries orient to the posterior segment of the eye further distributing to choriocapillaris (Forrester, 2008). The capillaries below the retinal pigment epithelium are densely fenestrated with small pores (Cavallotti et al., 2005; Guymer et al., 2004; Sugita et al., 1982). The diameter of the pores is only 75–85 nm that may limit the size of the particles passing through (Guymer et al., 2004).

Since the pivotal studies of Bangham (Bangham et al., 1965), liposomes have been investigated in drug delivery. They have been investigated also for topical ocular and intravitreal drug delivery (Kawakami et al., 2001; Law et al., 2000; Wang et al., 2012). Topical administration for posterior segment delivery is the greatest challenge and opportunity of ocular liposomal drug delivery. It has been shown that liposomal drug can reach the choroid, RPE and retinal ganglion cells after intravitreal injections and neural retina and ganglion cells after topical eye drop administration, respectively (Davis et al., 2014; Masuda et al., 1996). Lately, also nanosized lipid emulsions have been utilized a topical drug delivery system to the retina (Ying et al., 2013). However, delivery to the RPE and the route of transport from the surface of the eye to the back remains to be studied. Also large differences in delivery were noted between liposome types. Liposome formulation variables might be used to tune the delivery properties to the ocular tissues. It is very unlikely that liposomes could permeate in the tight corneal barrier. Conjunctiva and would be more probable route of penetration. Paracellular transport through the conjunctiva is possible for particles up to diameter of 5.5 nm and any particles larger than that would be carried via endocytosis pathways (Horibe et al., 1997). Conjunctiva can take up nanoparticles with diameter of 300 nm (Enriquez de Salamanca et al., 2006) and permeation of liposomes through the conjunctiva has been shown *in vitro* (Kompella et al., 1998). After passing through the conjunctiva, the drugs can reach the back of the eye via diffusion through sclera and vitreous or more directly via blood circulation to the choroid (Forrester, 2008; Hughes et al., 2005). Blood vessel mediated liposomal delivery to the posterior segment can be achieved only if the liposomes are able to distribute to the ocular tissues. For this purpose, the size of the liposomes is critically important due to the limited size of choroidal and conjunctival fenestrations (Guymer et al., 2004; Krachmer et al., 2011).

To achieve small liposomal size, we developed methods utilizing microfluidizers. The device drives liquid with high pressure (even 30,000 psi) through an interaction chamber. Thereafter, the liquid is collected or re-cycled through the microfluidizer. Particle size reduction is due to the shear forces at the chamber walls (Z-type chamber) or shear forces and the particle collisions (Y-type chamber) when high speed fluid streams with opposite directions merge. Microfluidizers have been used to process emulsions (Mahdi Jafari et al., 2006), polymer particles (Bodmeier and Huagang, 1990; Sani et al., 2009), crystalloid solids (Siqueira et al., 2009) and liposomes (Takahashi et al., 2009; Thompson and Singh, 2006). As a method of producing liposomes, microfluidizing has benefits of low polydispersity (Mahdi Jafari et al., 2006), continuous flow of solvent without a danger of clogging, suitability to heat sensitive drugs, and ease of scale up to larger volumes (Sorgi and Huang, 1996). The final particle size depends on the main process parameters, such as the pressure, temperature, and passage times through the microfluidizer (Mayhew et al., 1984; Saheki et al., 2012; Thompson and Singh, 2006; Washington and Davis, 1988).

Microfluidizer methods were optimized to produce small sized (<85 nm) liposomes. In order to elucidate the importance of liposomal diameter, we determined the effect of the liposomal size on their *in vivo* distribution in rat eyes after topical administration as eye drops. We demonstrate liposomal targeting to the retinal pigment epithelium with small transferrin conjugated liposomes.

2. Materials and methods

2.1. Materials

Hydrogenated soy L-α-phosphatidylcholine (HSPC) was a gift from NOF CORPORATION (Tokyo, Japan). 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), egg L-α-phosphatidylcholine (Egg PC), 1,2-distearoyl-sn-