Ultrasound in Med. & Biol., Vol. 39, No. 4, pp. 638–646, 2013

0301-5629/$ - see front matter

ELSEVIER

http://dx.doi.org/10.1016/j.ultrasmedbio.2012.11.010

● *Original Contribution*

ULTRASOUND-ENHANCED DELIVERY OF ANTIBIOTICS AND ANTI-INFLAMMATORY DRUGS INTO THE EYE

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(*Received* 8 *June* 2012; *revised* 6 *November* 2012; *in final form* 12 *November* 2012)

Abstract—Delivery of sufficient amounts of therapeutic drugs into the eye is often a challenging task. In this study, ultrasound application (frequencies of 400 KHz to 1 MHz, intensities of 0.3–1.0 W/cm^2 and exposure duration of 5 min) was investigated to overcome the barrier properties of cornea, which is a typical route for topical administration of ophthalmic drugs. Permeability of ophthalmic drugs, tobramycin and dexamethasone and sodium fluorescein, a drug-mimicking compound, was studied in ultrasound- and sham-treated rabbit corneas *in vitro* using a standard diffusion cell setup. Light microscopy observations were used to determine ultrasound-induced structural changes in the cornea. For tobramycin, an increase in permeability for ultrasound- and sham-treated corneas was not statistically significant. Increase of 46%–126% and 32%–109% in corneal permeability was observed for sodium fluorescein and dexamethasone, respectively, with statistical significance ($p < 0.05$) achieved at all treatment parameter combinations (compared with sham treatments) except for 1-MHz ultrasound applications for dexamethasone experiments. This permeability increase was highest at 400 kHz and appeared to be higher at higher intensities applied. Histologic analysis showed structural changes that were limited to epithelial layers of cornea. In summary, ultrasound application provided enhancement of drug delivery, increasing the permeability of the cornea for the anti-inflammatory ocular drug dexamethasone. Future investigations are needed to determine the effectiveness and safety of this application in *in vivo* long-term survival studies. (E-mail: mnabili@gwu.edu)

Key Words: **Therapeutic ultrasound, *In vitro*, Drug delivery, Cornea, Ocular diseases, Sonophoresis.**

INTRODUCTION

Millions of people suffer from variety of ocular diseases, which in some cases lead to vision impairment and eventually blindness (Clark et al. 2003; Friedman et al. 2002). Delivery of drugs at therapeutic levels in treatment of various ocular diseases is a challenge because of specific structure, defense mechanisms and physical barriers of the eye (Short 2008). Common approaches for drug administration to the eye include but are not limited to systemic administration, intravitreal injections, ocular implants and topical administration (Gaudana et al. 2010; Short 2008). Systemic drug delivery is inefficient because of different eye-blood barriers, including the barriers that prevent delivery of compounds into the anterior chamber of the eye (Davis et al. 2004; Gaudana et al. 2010) and into the posterior or back of the eye (Ali et al. 2008; Davis et al. 2004). Furthermore, systemically applied ophthalmic drugs can carry a risk of severe adverse effects as the drug enters the systematic circulation (Davies 2000; Gaudana et al. 2010). The intravitreal injection is the most direct and effective way of ocular drug delivery; however, it carries a potential risk of severe adverse effects including cataract, retinal detachment and ocular hemorrhage (Bartlett et al. 1984; Gaudana et al. 2010; Janoria et al. 2007; Short 2008). The easiest way to deliver the drug inside the eye is via topical administration (Cheung et al. 2010), which is used to treat both the eye surface and intraocular conditions (Reddy et al. 1996) because of its noninvasive nature and high patient compliance (Gaudana et al. 2010). This method has several advantages: therapeutic effects are localized and unwanted systemic effects are significantly reduced, and it is a fairly convenient and painless method of drug administration (Davies 2000). However, drug delivery

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using this method is adversely influenced by eye drop immediate spillage from the eye, tear removal, and ocular barriers such as cornea and sclera barriers (Ali et al. 2008; Gaudana et al. 2010) that cause limited penetration of the drug into the eye (Short 2008).

The cornea is a preferred route for topical administration of drugs (Ahmed et al. 1987b; Doane et al. 1978) and consists of three main layers: epithelium, stroma and endothelium (Gaudana et al. 2010; Gwon 2008). Unfortunately, corneal layers represent a major barrier for delivering drugs, which makes it difficult for therapeutic compounds to reach the target ocular tissues (Davies 2000). The epithelial layer is the main barrier for hydrophilic drugs, whereas the stroma mostly acts as a barrier for lipophilic drugs (Davies 2000; Ke et al. 1999). In most cases, the amount of drug that can penetrate through the cornea is less than 10% (Geroski and Edelhauser 2000; Ke et al.1999; Schoenwald 1997; Short 2008), and achieving a twofold to threefold increase in trans-corneal drug delivery is considered clinically significant (Sasaki et al. 1995).

The objective of our study was to investigate ultrasound enhancement of the delivery of ocular drugs (the antibiotic tobramycin and the steroid dexamethasone) through the cornea in a rabbit eye model *in vitro*. The corneal permeability of sodium fluorescein, a drug-mimicking compound, was also investigated using a limited set of parameters for comparison with published studies. Ultrasound has been shown to enhance the delivery of lytic agents into thrombi and anticancer drugs into cells (Abe et al. 2002; Lawrie et al. 1999; Mitragotri 2005; Tachibana and Tachibana 2001). It has also been used for gene delivery (Kowalczuk et al. 2011) into a variety of cells, such as myocardial and endothelial cells (Kodama et al. 2006; Miller et al. 2002; Taniyama et al. 2002). Furthermore, the application of ultrasound for delivery of drugs into the skin has been one of extensively investigated research areas with promising results (Mitragotri et al. 1995; Tang et al. 2001). Barrier properties of the cornea have also been shown to be modified by the application of ultrasound (Cherkasov et al. 1974; Gvarishvili and Dushin 1999; Tsok et al. 1990). The enhancement of drug delivery through the cornea by ultrasound (phonophoresis) was used clinically in the treatment of eye diseases (Cherkasov et al. 1974; Filippenko and Tretiak 1989; Marmur et al. 1979; Tsok 1979) in which phonophoresis was shown to have a positive effect on the outcome of the diseases of the eye anterior segment, such as keratitis and corneal opacities. Phonophoresis also caused faster healing of corneal ulcers and wounds, and faster resolving of corneal inflammation in patients (Egorov et al. 1995; Iakimenko et al. 1989; Marmur et al. 1979; Tsok et al 1979).

Results of our previous study *in vitro* showed that the exposure of the cornea to 880-KHz ultrasound increased the corneal permeability for sodium fluorescein, a small hydrophilic dye (Zderic et al. 2004a). In the study reported here, our goal was to test clinically relevant compounds that are currently used in the treatment of corneal infections and inflammations (*i.e.,* tobramycin and dexamethasone) and to test a range of frequencies and intensities to find the ones that might provide effective and safe drug delivery through the cornea.

MATERIALS AND METHODS

Compounds used in our experiments included the ophthalmic drugs tobramycin and dexamethasone sodium phosphate, and the drug-mimicking compound sodium fluorescein. This drug-mimicking compound was used in the initial stages of the study at specific parameters for comparison with our previously published results.

Tobramycin ophthalmic solution 0.3% (Bausch and Lomb Inc., Tampa, FL, USA) is a topical ophthalmic antibiotic formulation prepared specifically for therapy of external infections. This drug is a clear solution and is highly hydrophilic (DiCicco et al. 2003), with a molecular weight of 467.52 D according to the data sheet for this drug. Sodium fluorescein (Sigma-Aldrich, St. Louis, MO, USA) is an orange hydrophilic dye with a molecular weight of 376.27 D (according to the data sheet for this compound) and is used as a model for drugs that penetrate poorly through the cornea (Ke et al. 1999). Sodium fluorescein was used to make a 0.25% solution in Dulbecco phosphate-buffered saline (DPBS, D4031, Sigma-Aldrich), which is a balanced salt solution with inorganic ions and glucoses. Dexamethasone sodium phosphate 0.1% (Bausch and Lomb Inc.) is a topical steroid solution used to suppress inflammatory responses. This drug is a clear solution with hydrophilic properties and a molecular weight of 516.41 D.

Excised eyes of adult New Zealand White rabbits were used in our experiments. Because of their relative similarity to human eyes, rabbit eyes have been used as the standard model for ophthalmic research (Cheung et al. 2010; Gwon 2008; Ke et al. 1999). The size of the eyeballs in rabbits is smaller in comparison to human eyes with anteroposterior size of 16–19 mm in rabbits and 24 mm in humans. The thickness of a rabbit cornea is 0.3–0.4 mm in center of the cornea and 0.45 mm in its periphery (Gwon 2008). These measurements in human eyes are 0.5 mm and 0.7–0.10 mm, respectively. Rabbit corneal epithelium is $\sim$30–40 μm in thickness (Gwon 2008), which is thinner than the 50–60-μm corneal thickness of a human eye (Snell and Lemp 1998). The rabbit cornea consists of one row of columnar