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● *Original Contribution*

SONOPHORESIS USING ULTRASOUND CONTRAST AGENTS FOR TRANSDERMAL DRUG DELIVERY: AN *IN VIVO* EXPERIMENTAL STUDY

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Abstract—Sonophoresis temporally increases skin permeability such that various medications can be delivered noninvasively. Previous sonophoresis studies have suggested that cavitation plays an important role in enhancing transdermal drug delivery (TDD). In this study, the feasibility of controlled cavitation using ultrasound contrast agents (UCAs) at high frequency was explored through *in vivo* experiments in a rat model. Two commercially available UCAs, SonoVue® and Definity®, were used at 2.47 MHz and 1.12 MHz, respectively. Fluorescein isothiocyanate (FITC)-dextran with 0.1% UCA was used as the drug to be delivered through the skin. Ultrasound with a 10 ms pulse and a 1% duty cycle at 1 MPa acoustic pressure for 30 min was applied in all sonication sessions. The efficacy of sonophoresis with UCAs was quantitatively analyzed using an optical imaging system that was used to count photons emitted from fluorescein. The results showed that the proposed sonophoresis method significantly improved drug penetration compared with the traditional sonophoresis method with 4 kD, 20 kD and 150 kD FITC-dextrans at 1.12 MHz, and with 4 kD and 20 kD FITC-dextrans at 2.47 MHz. Sonophoresis for TDD was performed more effectively with the aid of UCAs. Sonophoresis with UCAs has excellent potential for broad applications in drug delivery for diseases requiring the chronic administration of medications such as diabetes. (E-mail: jongbums@yonsei.ac.kr)

Key Words: **Transdermal drug delivery, Ultrasound contrast agent, Cavitation, Sonophoresis, Noninvasive drug delivery.**

INTRODUCTION

Transdermal drug delivery (TDD) has considerable advantages compared with oral delivery routes in that it circumvents gastrointestinal degradation and first-pass metabolism through the liver (Prausnitz 2004; Lavon and Kost 2004). However, the use of TDD has been limited due to low permeability of the skin. The epidermis is the functional barrier of the outermost part of the skin and protects the human body as a first-line defense against foreign substances such as bacteria and toxins (Naik et al. 2000). The stratum corneum (SC) is the outermost layer of the epidermis that acts as the prominent epidermal permeability barrier (Choi et al. 2003). The SC is composed of corneocytes and intercellular lipids, and controls the percutaneous absorption of water, electrolytes and drugs (Elias 1983). Although the skin plays a critical role in human health, the SC is also the main obstacle to TDD. Hence, TDD systems focus on temporally disturbing the SC to administer medications for clinical applications without causing significant damage to the skin structure.

A number of methods have been proposed to temporally increase SC permeability. Chemical enhancers such as Azone® (Echo Therapeutics, Philadelphia, PA, USA) (1-dodecy-lazacycloheptan-2-one with molecular formula of $C_{12}H_{35}NO$, MW: 281.48) and soft enhancement of percutaneous absorption (SEPA) agent (2-n-nonyl-1,3-dioxolane with molecular formula of $C_{12}H_{24}O_2$, MW: 200.32) are known to increase skin permeability without causing irritation (Williams et al. 2004; Prausnitz et al. 2008). These chemical enhancers have been used successfully to deliver small molecules but they have limited impact for delivering hydrophilic compounds or macromolecules (Guy et al. 2003; Prausnitz et al. 2004).

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Iontophoresis utilizes low level current to increase the mobility of charged molecules across the skin (Singh et al. 1994; Li et al. 2002) and provides an electrical driving force to transport molecules across the SC. Although iontophoresis is able to deliver charged drugs through the SC, it is only applicable to small molecules up to a few thousand Daltons (Prausnitz and Langer 2008). Microneedles can be used to create microscale channels through the skin but this method is painful to recipients (Prausnitz 2004). Similarly electrophoresis, which uses multiple short pulses of high voltage electricity, can be affective but it is also painful (Prausnitz et al. 1993; Banga et al. 1999). Sonophoresis, which involves the use of ultrasound to provide mechanical force, is painless and functions independently of drug electrical characteristics (Ogura et al. 2008).

Recent research in sonophoresis has indicated that MHz range ultrasound has little effect on skin permeability even though 2–10 MHz is the most used frequency range in medical ultrasound imaging. On the other hand, low frequency (*e.g.*, $<$100 kHz) ultrasound is better suited for the transdermal transport of various drugs such as insulin, erythropoietin and interferon (Tachibana 1992; Mitragotri et al. 1995; Sinha et al. 2000). Given that heat deposition is dominant at high frequency and cavitation effects are prominent at low frequency, the main mechanism for sonophoresis delivery is thought to be cavitational, and not thermal, effects (Tang et al. 2002; Tezel et al. 2003; Lavon et al. 2004).

The source of cavitation is believed to be small bubbles trapped in the skin during topical application of a drug solution and oscillation of these bubbles according to the applied ultrasound field (Bommannan et al. 1992). Since bubbles oscillate in asymmetric boundary conditions, microstreaming and asymmetric bubble collapse can occur (Mitragotri et al. 1996). Microstreaming around the trapped bubbles causes shear stress directly on the SC layer such that overall skin permeability is increased (Baker et al. 2001; Collis et al. 2010). Asymmetric bubble collapse can induce jet streaming into the skin layer so that direct drug transport can occur (Tezel et al. 2003). Therefore, an increase of cavitation is a logical way to increase skin permeability in sonophoresis.

Adopting a low frequency ultrasound field is one way to increase existing bubbles in cavitation. When the frequency is low enough, it is assumed to be quasi-static field to sufficiently small bubbles. If small bubbles can rapidly grow and collapse when adequately exposed to quasi-static rarefractional pressure, these bubble activities can be related to asymmetric bubble collapse (Ciuti et al. 2000; Aganin et al. 2002). Another approach to increasing cavitation activity is to use a narrow bubble size distribution. If specific bubbles with a certain resonance frequency can be added uniformly, cavitation activity can be generated more uniformly and predictably over the skin area. Fortunately, ultrasound contrast agents (UCAs) are a group of microbubbles with a size distribution that is controlled to within a certain range such that they have MHz range resonance frequency (Schneider 1999a; Schneider 1999b; Cosgrove et al. 2006).

In previous work, we demonstrated that sonophoresis is more effective in drug delivery in the presence of UCAs in the high frequency range (*e.g.,* $>$1 MHz) and validated this method via *in vitro* experiments on porcine skin (Park et al. 2010). In the present study, we further evaluated the efficacy of sonophoresis with UCAs through *in vivo* experiments in a rat model. Two types of commercial UCAs, Definity® (Bristol-Myers Squibb Medical Imaging, No. Billerica, MA, USA) and SonoVue® (Bracco Diagnostics Inc., Milan, Italy), were used along with MHz range ultrasound according to the specific resonance frequencies of the individual UCAs. Three FITC-dextrans of different molecular weights were used as the target drug and delivered through the skin. The results were quantitatively analyzed using an optical imaging system. Histologic analyses were also conducted upon experiment completion to evaluate the level of skin damage caused by sonophoresis.

MATERIAL AND METHODS

Skin preparation

In vivo experiments were conducted using Sprague Dawley rats (male, 4 to 8 weeks of age, weight ranging from 240 to 270 g, Orient Bio Inc., Sungnam, Korea). The rats were anesthetized *via* isoflurane inhalation (1%–2% in 100% oxygen, Forane Solution; Choongwae Pharma Co., Seoul, Korea) on a heating pad and two exposure areas were shaved on their backs. Hair removal gel cream (Veet Rasera^{TM} for sensitive skin; Reckitt Benckiser, Mississauga, Canada) was applied to the shaved spot followed by cleaning with gauze soaked in warm water. All experiments were conducted in accordance with the Guidelines for Animal Experiments outlined by the Association for the Assessment and Accreditation of Laboratory Animal Care International (Bayne et al. 1998).

Target drugs and UCAs

Dextran is a complex molecule with varying lengths of glucose molecules. Dextran is hydrophilic and generally cannot permeate the skin. In addition, dextrans of various sizes are readily available commercially. Hence, dextran is widely used as a test material for the validation of TDD efficacy (Lombry et al. 2000; Wang et al. 2005; Wu et al. 2006). FITC-dextran (Sigma Chemical Co., St. Louis, MO, USA) was used as the target drug in our experiments. Since FITC-dextran is labeled with a fluorescent substance,