ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES 10 (2015) 415–424

HOSTED BY

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ajps

Original Research Paper

Transdermal delivery of fluorescein isothiocyanate-dextrans using the combination of microneedles and low-frequency sonophoresis

Boonnada Pamornpathomkul [a,b], *Sureewan Duangjit* [b,c], *Suvida Laohapatarapant* [a,b], *Theerasak Rojanarata* [a,b], *Praneet Opanasopit* [a,b], *Tanasait Ngawhirunpat* [a,b,*]

[a] *Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand*

[b] *Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand*

[c] *Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand*

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form 6 May 2015
Accepted 8 May 2015
Available online 9 July 2015

Keywords:
Microneedle
Sonophoresis
Fluorescein isothiocyanate-dextran
Transdermal drug delivery

ABSTRACT

This study aimed to evaluate the patient-friendly methods that are used in the delivery of hydrophilic macromolecules into deep skin layers, in particular, the combination of microneedles patch (MNs patch) and low-frequency sonophoresis (SN). The hydrophilic macromolecule drug fluorescein isothiocyanate (FITC)-dextrans (FD-4: MW 4.4 kDa) was used as the model drug in our experimental design. In this study, excised porcine skin was used to investigate and optimize the key parameters that determine effective MNs- and SN-facilitated FD-4 delivery. *In vitro* skin permeation experiments revealed that the combination of MNs patch with SN had a superior enhancing effect of skin permeation for FD-4 compared to MNs alone, SN alone or untreated skin, respectively. The optimal parameters for the combination of MNs and SN included the following: 10 N insertion force of MNs, 4 W/cm^2 SN intensity, 6 mm radiation diameter of the SN probe, 2 min application time, and the continuous mode duty cycle of SN. In addition, vertical sections of skin, clearly observed under a confocal microscope, confirmed that the combination of MNs and SN enhanced permeation of FD-4 into the deep skin layers. These studies suggest that the combination of MNs and SN techniques could have great potential in the delivery of hydrophilic macromolecules into deep skin.

* *Corresponding author.* Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand. Tel.: +66 34 255800; fax: +66 34 255801.
E-mail address: ngawhirunpat_t@su.ac.th (T. Ngawhirunpat).

Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2015.05.002

1. Introduction

Transdermal drug delivery systems (TDDS) represent an attractive alternative form of drug delivery that minimizes and circumvents the limitations associated with the oral, parenteral, and inhalation methods of drug administration [1]. Transdermal delivery offers several potential advantages, including avoidance of hepatic first pass metabolism, reduction of the severe adverse effects on the gastrointestinal tract associated with oral drug administration, avoidance of drug level fluctuation and ease of drug delivery termination in the occurrence of toxicity. However, TDDS also face major challenges in circumventing the barrier function of the outermost layer of the skin, the stratum corneum (SC). The SC works against drug penetration into the skin and the systemic circulation of TDDS [2,3].

To overcome the SC barrier, several physical enhancement methods have been evaluated for the ability to increase skin permeation and allow for transdermal administration of water-soluble molecules and macromolecular drugs [4]. One of the interesting physical enhancement methods, the microneedles (MNs) array, is composed of small micron-sized needles which, when applied on the skin, disrupt the SC barrier by creating large aqueous microchannels that allow for the passage of molecules through the skin barrier without causing skin damage [5]. These abrasions provide physical pathways for the drug to permeate the skin in much higher concentrations than would be normally observed by topical administration. MNs design can be commonly divided into 3 types: solid MNs, hollow MNs, and biodegradable MNs. Different materials have been used in the fabrication of MNs, including silicon rubber, stainless steel, titanium, glass, polysaccharide, and numerous polymers [6,7]. Moreover, MNs are of interest primarily because they offer the promise of less invasive and painless drug delivery as MNs do not penetrate the papillary layer of dermis where nerve endings are located [7]. In addition to increasing transdermal delivery, a combination of physical enhancement methods should also reduce the level of the enhancers required to achieve the desirable drug flux [8]. Yan et al. combined the MNs array with in-skin electroporation (in-skin EP), which creates new permeation pathways in the SC, thereby enhancing skin permeability. The combination of MNs and in-skin EP was considered to have excellent synergistic effects for FD-4 skin permeation compared to MNs alone or conventional EP [9].

Recently, another physical method, sonophoresis (SN), has also been shown to effectively deliver various types of drugs regardless of their electrical characteristics [10,11]. SN is a promising approach that involves the use of ultrasound energy for delivery of molecules that are normally impermeable to deep skin layers. Several proposed mechanisms of SN have been reported; these include acoustic cavitation effects, the formation and collapse of gaseous cavities in the SC barrier [12] and thermal effects that are due to ultrasound wave attenuation. An increase in the temperature of tissues can increase skin permeability [13]. It has been suggested that SN-based enhancement in skin permeability depends on ultrasound parameters, such as frequency, intensity, duty cycle, radiating diameter of the transducer, and the duration of ultrasound application [14]. SN application, with ultrasound energy at frequencies in the range of 20 kHz–16 MHz and intensities up to 14 W/cm^2, enhances skin permeation of high molecular-weight drugs. In particular, transdermal enhancement is significantly higher at low-frequency regimens (20 kHz–100 kHz) compared to induction by high-frequency ultrasound (1 MHz–16 MHz) [15].

Despite their different mechanisms, MNs and SN can be coupled through the formation of integrated systems. The use of both methods can enhance the skin penetration of molecules by creating new permeation pathways and increasing the duration of the pore opening for higher drug permeation. Chen et al. evaluated the synergistic effect of SN combined with MNs in the delivery of calcein and bovine serum albumin. The results showed that the combination of SN and MNs greatly enhanced transdermal drug delivery rates compared to passive diffusion and either MNs or ultrasound alone [16]. Furthermore, the maximum application strength of the enhancers is typically limited by safety restrictions. The combination of two or more physical enhancement methods can achieve the desired enhancement with significantly reduced strength of the individual enhancers. Hence, a combination of physical enhancement methods not only improves the total enhancement but also increases the safety of enhancer use [8]. However, the details of factors and optimum conditions for the combination of MNs and SN application for skin permeation in TDDS are rarely studied.

In this study, we examined the effect of various parameters of MNs and low-frequency SN on fluorescein isothiocyanate (FITC)-dextrans (FD-4) skin permeation. The minimum effective condition for minimal invasiveness of the combination of MN and SN was our desirable achievement, and the safety issues are important awareness for this combination study. Our evaluation included the following: force insertion of the MNs, the intensity, application time, and diameter transducer of the SN, and ultrasound duty cycle (ratio of ultrasound time duration). FD-4, a hydrophilic macromolecule with a high molecular weight (4.4 kDa), was selected as our model drug because it has characteristically poor skin penetration [9]. The effect of MNs and low-frequency SN on skin permeation enhancement was determined by *in vitro* skin permeation experiments. Skin morphology after treatment with MNs and SN was observed using confocal laser scanning microscopy.

2. Materials and methods

2.1. Materials

Fluorescein isothiocyanate (FITC)-dextrans (FD-4; average molecular weight, 4.4 kDa) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Fabrication of microneedles patch

The solid MNs patch was fabricated from stainless steel, 32-gauge acupuncture needles (0.25 × 30 mm, DongBang Acupuncture Inc., Boryeong, Korea) and a silicone sheet