Permeation of sumatriptan succinate across human skin using multiple types of self-dissolving microneedle arrays fabricated from sodium hyaluronate

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ABSTRACT

Available formulations of sumatriptan succinate (SS) have low bioavailability or are associated with site reactions. We developed various types of self-dissolving microneedle arrays (MNs) fabricated from sodium hyaluronate as a new delivery system for SS and evaluated their skin permeation and irritation in terms of clinical application. In vitro permeation studies with human skin, physicochemical properties (needle length, thickness and density), and penetration enhancers (glycerin, sodium dodecyl sulfate and lauric acid diethanolamide) were investigated. SS-loaded high-density MNs of 800 \(\mu\)m in length were the optimal formulation and met clinical therapeutic requirements. Penetration enhancers did not significantly affect permeation of SS from MNs. Optical coherence tomography images demonstrated that SS-loaded high-density MNs (800 \(\mu\)m) uniformly created drug permeation pathways for the delivery of SS into the skin. SS-loaded high-density MNs induced moderate skin irritations in rats, but the skin recovered within 72 h of removal of the MNs. These findings suggest that high-density MNs of 800 \(\mu\)m in length are an effective and promising formulation for transdermal delivery of SS. To our knowledge, this is the first report of SS permeation across human skin using self-dissolving MNs.

Introduction

Sumatriptan succinate (SS), a selective serotonin 5-hydroxytryptamine (5-HT) agonist that acts on 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} subtype receptors, is the most frequently prescribed drug among the triptans, for the treatment of migraine headaches [1]. Currently available formulations of SS, including oral formulations, nasal sprays and subcutaneous injection formulations, are associated with several limitations that can affect treatment outcomes, such as difficulty with oral medication owing to nausea and vomiting that often accompany migraine headaches, as well as the low systemic bioavailability of oral formulations and nasal sprays (15% and 17%, respectively). Subcutaneous injections are associated with skin site reactions and poor patient compliance owing to the invasive nature of injections and the pain they elicit [2–7]. An effective alternative method for delivering SS into the systemic circulation and overcoming the limitations of current delivery methods will improve migraine treatment strategies, thus benefitting patients.

Transdermal drug delivery allows permeation of drugs through the skin and into the systemic circulation, thus avoiding degradation in the gastrointestinal tract and hepatic first-pass metabolism; hence, it is an attractive route of administration. However, transdermal drugs are limited to a few hydrophobic, low-molecular weight compounds because of the properties of the stratum corneum, the outermost layer of the skin [8,9]. Due to the relatively high hydrophilicity of SS, absorption through the skin is difficult [10,11]. Previous studies employing iontophoretic transdermal technology (Zecuity\textsuperscript{®}, NuPath Inc., Conshohocken, PA) demonstrated that this system successfully delivered an amount of SS sufficient for acute treatment of migraine headaches in humans [12,13].

Recently, microneedle arrays (MNs), a novel, painless and minimally invasive drug delivery system, have attracted significant attention [14–16]. MNs are composed of micron-size needles that can disrupt the stratum corneum and enhance skin permeability of low-molecular weight drugs, proteins and influenza vaccines [17–19]. MNs have been fabricated in a wide range of designs from various materials, including silicon [20,21], metal [18,22–24] and glass [25]. Furthermore, MNs made of biodegradable or bio-compatible polymers [17,19,26–29] and water-soluble carbohydrates [30–33] are capable of completely dissolving in the skin and therefore, do not leave sharp biohazardous waste after use, unlike silicon-, metal- or glass-based MNs. We recently investigated SS-loaded, self-dissolving MNs fabricated from sodium hyaluronate and demonstrated efficient transdermal delivery of SS in rats [34]. Hyaluronate, which is a major component of skin, was found to produce MNs with high biocompatibility and resistance to deformation [33,34]. Despite the promising characteristics of SS-loaded, self-dissolving MNs, the relationship between the physicochemical characteristics of hyaluronic acid-based MNs, such as needle length, thickness and density, and skin permeation by the loaded drug remains to be elucidated.

The aim of this study was to assess the clinical potential of hyaluronate-based MNs by investigating the influence of their physicochemical characteristics (needle length, thickness and density) and penetration enhancers on human skin permeation of SS using Franz diffusion cells. In addition, the penetration characteristics of SS-loaded high density MNs in human skin were evaluated by optical...
coherence tomography (OCT). Finally, in vivo skin irritation of SS-loaded high-density MNs was assessed in rats by the Draize test.

**Materials and methods**

**Materials**

SS was purchased from Viwit Pharmaceutical Co., Ltd. (Shanghai, China). Sodium hyaluronate (Japanese Pharmacopoeia (JP) grade) was purchased from Kikkoman Biochemifa Company (Tokyo, Japan). L-((+))-Tartaric acid was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Concentrated cosmetic glycerin was purchased from Miyoshi Oil & Fat Co., Ltd. (Tokyo, Japan). Sodium lauryl sulfate was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Lauric acid diethanolamide (LD) was purchased from Kao Corporation (Tokyo, Japan). Acetonitrile and ammonium dihydrogen phosphate were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals and reagents were of analytical reagent grade.

Male Wistar rats (8-week-old, 250–270 g) were purchased from Japan SLC Inc. (Shizuoka, Japan). All animal experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Animal Experimentation Committee of the Kyoto Pharmaceutical University approved all protocols for animal experiments.

**Fabrication of SS-loaded MNs**

MN containing SS were fabricated using micromolding technology with sodium hyaluronate as the base material [33,34]. In brief, sodium hyaluronate was dissolved in distilled water, after which SS solution (with or without penetration enhancers) dissolved in 3% tartaric acid was added to the sodium hyaluronate solution and uniformly mixed. The resulting solution was cast in micromolds and dried in a desiccator at room temperature. After the micromolds dried completely, the polyethylene terephthalate (PET) adhesive tape was attached to the base plate for reinforcement. The SS-loaded MNs were removed from the micromolds and cut into circular sections with a diameter of 10 mm using a punch. MNs of different length, thickness and density were fabricated in the same manner using various micromolds.

**Improvement of transdermal delivery of SS from SS-loaded MNs in vitro**

In vitro skin permeation studies were performed using vertical Franz diffusion cells with a diffusion area of 3.14 cm². Human cadaver skin, for which all ethical committee approval and informed patient consent was obtained, was purchased from Biological Resource Center, Inc. (Phoenix, AZ). Subsequent studies were conducted in agreement with the Declaration of Helsinki. Informed patient consent and the approval of the Ethical Committee of Kyoto Pharmaceutical University were obtained. Residual subcutaneous fat and other extraneous tissues in human skin were trimmed to a thickness of 1000 μm using a surgical electric dermatome. SS-loaded MNs were inserted into the excised skin with an applicator and left in place for 5 min. Upon removal of the array, the treated site was immediately examined using a Gamynede model OCT microscope (Thorlabs GmbH, Munich, Germany) to observe the microchannels created by the MNs.

**Primary skin irritation after application of SS-loaded MNs in rats**

Primary skin irritation after the application of high-density SS-loaded MNs of 800 μm in length was evaluated by OCT following insertion into excised human skin. We selected OCT, rather than traditional histological sectioning and staining, in order to visualize drug permeation in real time after the removal of MNs, thus avoiding skin damage associated with freezing, which could alter the structure of the skin [35,36]. Residual subcutaneous fat and other extraneous tissues in human cadaver skin were trimmed to a thickness of 1000 μm using a surgical electric dermatome. SS-loaded MNs were inserted into the excised skin with an applicator and left in place for 5 min. Upon removal of the array, the treated site was immediately examined using a Gamynede model OCT microscope (Thorlabs GmbH, Munich, Germany) to observe the microchannels created by the MNs.

**Statistical analyses**

The results were evaluated using Student’s unpaired t-test or an analysis of variance with subsequent Tukey’s multiple comparison tests at a significance level of p < 0.05.

**Results**

**Fabrication of SS-loaded MNs**

Five types of MNs with different needle length, thickness, density and penetration enhancers were prepared. Figure 1 shows a typical micrograph of an MN sample. All needles were tapered, cone-shaped and arranged in a circular array with a diameter of 10 mm. MNs of 500 μm in length (basal diameter, 100 μm; tip diameter,
35 μm; interspacing between needles, 600 μm) and 800 μm in length (basal diameter, 150 μm; tip diameter, 35 μm; interspacing between needles, 600 μm) were designed to have approximately 200 needles per array. The MN samples containing penetration enhancers were prepared with the micromolds used to prepare the MNs of 800 μm in length. The thick MNs of 800 μm in length were designed to have approximately 200 needles per array (basal diameter, 170 μm; tip diameter, 45 μm; interspacing between needles, 600 μm). The high-density MNs of 800 μm in length were designed to have approximately 500 needles per array (basal diameter, 110 μm; tip diameter, 25 μm; interspacing between needles, 350 μm).

**Effect of needle length on skin permeation of SS**

The effect of the length of the MNs on skin permeation of SS is shown in Figure 2. The length of the MNs affected permeation of SS. MNs (800 μm) containing 9.8 mg SS/cm² showed approximately three-fold greater SS permeation into the skin in comparison with that of the 500 μm MNs containing 6.3 mg SS/cm² (Figure 2A). Furthermore, as shown in Figure 2B, when the length of the needles was increased from 500 to 800 μm, the rate of SS permeation increased, while the maximum transdermal flux value significantly (p < 0.05) increased from 147.4 ± 2.7 to 386.8 ± 26.3 μg/cm²·h. These results suggest that transdermal permeation of SS can be improved by increasing the length of the MNs. Therefore, MNs of 800 μm in length were employed for subsequent studies.

**Effect of needle thickness on skin permeation by SS**

Further experiments were performed to improve transdermal delivery of SS from SS-loaded MNs of 800 μm in length. The cumulative permeation profiles of SS from the SS-loaded MNs are shown in Figure 3A. The mean cumulative amounts of permeated SS from
the standard (9.8 mg SS/cm²) and thick MNs (11.2 mg SS/cm²) at 4 h were 992.0 ± 34.8 µg/cm² and 975.4 ± 8 µg/cm², respectively. The cumulative amounts of permeated SS from the standard and thick MNs did not differ significantly (p > 0.05) over a 24-h period. Figure 3B shows that SS from both types of MNs rapidly permeated the skin, achieving maximum transdermal flux 1 h after application. These findings indicate that increasing the thickness of the MNs did not enhance skin permeation by SS.

**Effect of penetration enhancers on skin permeation by SS**

To improve transdermal delivery of SS from the synthesized MNs, penetration enhancers were added to the MNs at a concentration of 3%. Glycerin, sodium dodecyl sulfate (SDS), and lauric acid diethanolamide (LD) were selected for use as penetration enhancers in solutions containing 9.8 mg SS/cm². However, permeation of SS from the various MN solutions did not differ significantly (p > 0.05) over the experimental period (Figure 4). These findings indicate that increasing the thickness of the MNs did not enhance skin permeation by SS.

**Effect of needle density on the skin permeation of SS**

Figure 5A shows the cumulative amount of permeated SS after application of high-density MNs (11.0 mg SS/cm²) and standard MNs (9.8 mg SS/cm²) in vitro. SS rapidly permeated in all of the arrays. In comparison with the standard MNs, the high-density MNs produced a significantly greater amount of SS in the receiver compartments after 2 h (p < 0.05). In addition, the amount of permeated SS from the high-density SS-loaded MNs was 2150.6 ± 197.0 µg/cm² after 4 h, which was significantly higher (p < 0.05) than that obtained with the standard MNs (1040.4 ± 70.7 µg/cm²). Following rapid permeation of SS from the high-density MNs, transdermal flux decreased from 604.9 ± 73.7 µg/cm²/h to 184.1 ± 41.3 µg/cm²/h during the 24-h experimental period (Figure 5B). Taken together, the results of the in vitro skin permeation experiments indicate that high-density MNs of 800 µm in length provide permeation of sufficient amounts of SS through human skin.

**Skin penetration characteristics of high-density SS-loaded MNs**

Figure 6A shows a three-dimensional image (volumetric scan) of the human skin surface after insertion of MNs using OTC. Orderly micropores were created en face with a pattern similar to that of the array. Figure 6B shows a cross-sectional two-dimensional (2D) image of untreated human skin, which was intact prior to MN application. As shown in Figure 6C and D, distinct pathways were directly created across the skin after SS-loaded MNs were applied. Moreover, skin pierced with MNs showed penetration depths of approximately 250–300 µm, corresponding to insertion through the stratum corneum and epidermis and into the superficial dermis. These findings indicate that MNs are capable of creating uniform drug permeation pathways across the skin, allowing SS to be successfully delivered into the systemic circulation. These results also highlight the drug delivery mechanism of MNs following insertion into the skin.
Skin irritation after treatment with SS-loaded MNs was assessed by the Draize scoring criteria (Figure 7) at 1, 24 and 72 h after application. After removal of the MNs after 1 h, some erythema, and no edema were observed at the treated sites; however, erythema improved within 24 h, whereas the skin recovered from damage after 72 h. The primary irritation index (P.I.I.) was calculated to be 2.1 in rats treated with the high-density SS-loaded MNs of 800 μm in length, indicating moderate irritation (a P.I.I. between 2.0 and 4.9 indicates moderate irritation).

**Discussion**

To optimize transdermal delivery of SS from SS-loaded MNs fabricated from hyaluronate, we evaluated the effects of needle length, thickness, density and penetration enhancers on permeation of SS into human skin. Generally, the permeability of drugs in human skin is much lower than their permeability in rat skin, because of difference in the thickness and lipid composition of the stratum corneum [38]. In the present study, we examined human skin in skin permeation studies. The length of the MNs affected the transdermal delivery of SS; MNs with a needle length of 800 μm delivered a higher concentration of SS across the skin than that delivered by MNs with a length of 500 μm. These findings were consistent with a previous study by Oh et al., who showed that 500 μm MNs produced significantly enhanced transdermal calcein delivery in comparison with that of 200 μm MNs [19]. However, as shown in Figure 2A, approximately 1.1 mg/cm² SS was detected in the receptor compartment within 4 h, which was not adequate to induce a therapeutic response.

To obtain efficient skin permeation by SS, we tried to enhance MN drug loading by increasing the thickness of the needles. However, the permeation profiles of the standard and thick MNs did not differ significantly, perhaps because the aqueous SS
solution used to fabricate the SS-loaded MNs was saturated, rendering SS transport independent of its concentration. We also found that transdermal absorption of SS was not affected by the presence of absorption enhancers, perhaps because MNs directly disrupted the stratum corneum, the rate-limiting barrier to transdermal delivery while the enhancers were rapidly eliminated from the skin without any interaction with the stratum corneum.

The cumulative amount of permeated SS increased significantly as the number of needles was increased (Figure 5). Assuming that each MN created a perfectly circular hole with a diameter equivalent to that of its base, the total surface hole areas of the standard and high-density MNs were approximately 3.5 × 10^6 μm² and 4.8 × 10^6 μm², respectively. A 1.4-fold increase in total hole area was induced by increasing the numbers of needles per array. Consequently, enhancement of SS penetration may have been largely a result of increasing the surface area of the MN holes [26,32]. It is also evident from Figure 5A that high-density MNs delivered approximately 2.2 mg/cm² SS across the skin within 4 h, equivalent to 1.6 mg/cm² of sumatriptan (MW of SS, 413.5 Da; MW of sumatriptan monomer, 295.4 Da). A single dose of 6 mg of the available subcutaneous formulation of sumatriptan, Imiglurex® (GlaxoSmithKline, Brentford, Middlesex, UK), is used for the treatment of migraine headaches in the USA. However, the sufficient pharmacokinetics and pharmacological effect were obtained after low-dose subcutaneous injection (3 mg) in Japanese patients. Therefore, 3 mg was chosen for the subcutaneous injection formulation of sumatriptan (Imigran® injection) in Japan. With these findings in mind, we concluded that approximately 3.5 mg of transdermally delivered sumatriptan over 4 h is adequate for the treatment of migraine headaches in Japan, in contrast with the 6.5 mg dose of Zecuity® used over 4 h in the USA. In addition, for MNs of 800 μm in length, the maximum diameter of MN array suitable for clinical application is 2 cm, indicating that the maximum area of each circular array is limited to 3.14 cm². In the present study, it was found that approximately 1.6 mg/cm² of sumatriptan was delivered by MNs arrays of 2 cm in diameter within 4 h. It is assumed that MN arrays with a diameter of 2 cm would deliver approximately 5 mg of sumatriptan into the skin, considerably exceeding our target of 3.5 mg over 4 h for the treatment of migraine headaches in Japan. Therefore, high-density MNs fabricated from sodium hyaluronate are promising transdermal formulations that could be used to deliver SS into the systemic circulation of migraine patients.

In the evaluation of penetration characteristics after the application of MNs to human skin [39,40], we observed that distinct uniform micropores were created en face, confirming that the stratum corneum had been breached (Figure 6A). In addition, high-density MNs penetrated the epidermis and extended into the papillary dermis (Figure 6C and D). The depth of penetration was approximately 250–300 μm, which was considerably shorter than the total length of the MNs, because of skin elasticity and deformation. Even in the case of high drug loading, sodium hyaluronate-based MNs with a tapered, cone-shaped geometry and a length of 800 μm possessed mechanical strength sufficient to puncture the human skin and deliver SS into the upper dermis.

Consistent with the dissolving properties of the standard MNs fabricated from hyaluronate reported in our previous studies [33,36], the base plate of the MNs was almost completely dissolved within 24 h of application, with only the PET adhesive tape remaining (Figure S1). However, as shown in Figure 5A, approximately 65.5% of the total amount of SS in the MNs (11.0 mg/cm²) was detected in the receptor compartment (7.2 mg/cm²) within 24 h of application of the high-density SS-loaded MNs (800 μm). Some SS may have remained in the epidermis, dermis and stratum corneum; furthermore, a small fraction of the drug remained on the skin surface and PET tape. Yamamoto et al. reported that more drug permeated during in vivo steady-state transdermal absorption than during in vitro skin permeation because of the dermal blood supply and metabolic activity [41]. In our previous study [34], the bioavailability of SS from the standard MNs (800 μm) fabricated from hyaluronate was as high as approximately 90% in rats. Therefore, permeation of SS into the human skin from the high-density MNs fabricated from hyaluronate in vivo would be superior to that measured in vitro.

In the skin irritation study performed with the high-density SS-loaded MNs, moderate skin irritation was observed in rats, which showed skin irritation scores slightly higher than those of the placebo group from our previous study [35]. The increased number of needles may be parallel to the increased number of pores per unit area, resulting in slightly more severe skin irritation. However, consistent with the standard MNs fabricated from hyaluronate in our previous study [35,36], skin irritation caused by the high-density MNs was moderate and transient. Therefore, these results indicate that high-density MNs are safe; however, further studies are needed to assess their safety of the repeated application for a longer period of time for the clinical use.

**Conclusion**

Factors influencing in vitro skin permeation of SS were evaluated. Sufficient permeation by SS was obtained with high-density MNs of 800 μm in length. Even with high drug loading, the SS-loaded high-density MNs possessed mechanical strength sufficient to puncture the skin barrier and extend into the upper dermis. We successfully developed a new and promising transdermal delivery system for SS using high-density MNs fabricated from hyaluronate, which did not cause serious skin damage.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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