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The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin

Shu Liu [a], Mei-na Jin [a], Ying-shu Quan [a,b], Fumio Kamiyama [b], Hidemasa Katsumi [a], Toshiyasu Sakane [a], Akira Yamamoto [a,*]

[a] Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan
[b] CosMED Pharmaceutical Co. Ltd, Higashikujo Kawanishi-cho 32, Minami-ku, Kyoto 601-8014, Japan

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ABSTRACT

The aim of the present study was to develop novel insulin-loaded microneedle arrays (MNs) fabricated from hyaluronic acid (HA), and characterize their applicability in the transdermal delivery of insulin. The shape of MNs was observed via scanning electron microscopy. The characteristics of these novel insulin-loaded MNs, including hygroscopy, stability, drug release profiles, and dissolution properties, were evaluated from a clinical application point-of-view. Transepidermal water loss (TEWL) was measured to investigate the piercing properties of MNs, and the recovery of the skin barrier after the removal of MNs to confirm their safety. Additionally, the transdermal absorption of insulin from MNs was examined via an *in vivo* absorption study in diabetic rats. The length of MNs was 800 μm with a base diameter of 160 μm and a tip diameter of 40 μm. MNs were found to maintain their skin piercing abilities for at least 1 h, even at a relative humidity of 75%. After storing insulin-loaded MNs for a month at −40, 4, 20, and 40 °C, more than 90% of insulin remained in MNs at all temperatures, indicating that insulin is highly stable in MNs at these storage conditions. It was also found that insulin is rapidly released from MNs via an *in vitro* release study. These findings were consistent with the complete dissolution of MNs within 1 h of application to rat skin *in vivo*. Therefore, the novel HA MNs possess self-dissolving properties after their dermal application, and insulin appears to be rapidly released from these MNs. A significant increase in TEWL was observed after the application of MNs. However, this parameter recovered back to baseline within 24 h after the removal of MNs. These findings indicate that the transdermal transport pathway of insulin, which was created by the MNs, disappeared within 24 h, and that the skin damage induced by the MNs was reversible. Furthermore, a dose-dependent hypoglycemic effect and transdermal delivery of insulin were observed after a dermal treatment with insulin-loaded MNs *in vivo*. A continuous hypoglycemic effect was observed after 0.25 IU of insulin was administered to skin via MNs. Additionally, lower peak plasma insulin levels, but higher plasma insulin concentrations after 2 h, were achieved with 0.25 IU of insulin administered via MNs as compared to the subcutaneous administration of insulin of the same dose. Pharmacodynamic and pharmacokinetic parameters indicated that insulin administered via MNs was almost completely absorbed from the skin into the systemic circulation, and that the hypoglycemic effect of insulin-loaded MNs was almost similar to that of the subcutaneous injection of insulin. These findings indicate that the novel insulin-loaded MNs fabricated from HA are a very useful alternative method of delivering insulin via the skin into the systemic circulation without inducing serious skin damage. Therefore, HA MNs may be an effective and safe method of transdermal insulin delivery in the clinic.

1. Introduction

Insulin medication is currently effective in the treatment of Type I diabetes. Due to the poor absorption or enzymatic degradation of insulin in the gastrointestinal tract and liver, the subcutaneous route has been so far the preferred method of insulin administration. However, this route is associated with poor patient compliance due to the pain caused by injection and risk of inflammation and infection. Consequently, minimally invasive and comfortable routes, including pulmonary, nasal, buccal, and transdermal routes, have been investigated as alternative delivery routes of insulin [1–6]. However, the absorption of insulin from these routes into the systemic circulation is still poor compared to the subcutaneous injection route, and thereby, a more effective method of administration is warranted for the delivery of insulin, as well as other peptide and protein drugs, into the systemic circulation.

Recently, attention has been paid to the possibility of using MNs in delivering insulin into the skin. As a novel and minimally invasive approach, MNs are capable of creating superficial pathways across the

* Corresponding author. Tel.: +81 75 595 4661; fax: +81 75 595 4761.
E-mail address: yamamoto@mb.kyoto-phu.ac.jp (A. Yamamoto).

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skin for small drugs, macromolecules, nanoparticles, or fluid extractions to achieve enhanced transdermal drug delivery [7–10]. Needles of micrometer dimensions are long enough to penetrate the stratum corneum and provide precise penetration depth under the skin by controlling the length of the needles [11]. Their sharp tips are short enough to reduce damage to skin nerves and pain [12,13], and narrow enough to induce minimal trauma and reduce the opportunities for infections to develop during insertion [14]. This method combines the efficacy of conventional injection needles with the convenience of transdermal patches, while minimizing the disadvantages of these administration methods [15].

In previous attempts at MN-aided insulin delivery, hollow and solid MNs have been shown to enhance skin permeation of insulin via *in vitro* [8,16,17] and *in vivo* studies [8,17–22]. Hollow MNs have been used to facilitate diffusion or pressure-driven flow of insulin through a central lumen [8,17,20], whereas solid MNs have been used to pierce the skin prior to the application of an insulin solution so as to deliver pharmacologically active insulin to diabetic rats or mice [19,23]. These reports have implied that MN-aided delivery is a possible route of insulin administration. However, the use of MNs is associated with a number of problems. In the case of hollow MNs, specialized devices are required to control either the retraction distance of MNs after insertion in order to increase the microinjection flow rate [17] or pressure necessary to drive the flow of the liquid drug into the skin to make the whole system workable [8,20]. Conversely, solid MNs require a two-step application process, and the delivery of insulin appears to be inefficient in this manner [19,23]. Furthermore, these MNs are not applicable for rapid distribution or self-administration, and are fabricated from silicon, glass, or metal, which may be unsafe, if the needles broke and were retained in the skin after application.

To date, two strategies have been developed to overcome these shortcomings in MN-aided delivery. The first strategy is the use of biodegradable materials, such as polymers, including poly-lactide-*co*-glycolide acid, polylactic acid, polyglycolic acid and polycarbonate [24–26], or water soluble materials, such as carbohydrates, including maltose [27–29], galactose [30], and dextrin [21,31], to fabricate high biocompatible and biodegradable solid MNs. The other strategy is to fabricate drug-loaded MNs, which contain drugs within the needle matrix, and thereby provide a simple one-step application process that is efficient in drug delivery. However, the abovementioned biodegradable materials are not suitable for fabricating insulin-loaded MNs. Specifically, during the fabrication of MNs consisting of biodegradable polymers or maltose [24–29], a heating step is required, which may cause the breakdown of heat-sensitive drugs, such as insulin, and in turn, eradicate their pharmacological activities. Additionally, polymeric needles possess slow-degrading properties, and these characteristics can retain insulin in the skin for a long period of time. Thus, they are not suitable for rapid drug release necessary for insulin delivery. Unlike polymeric needles, maltose and galactose MNs are readily soluble and dissolve in skin within minutes. However, in a wet environment exceeding humidity levels of 43% to 50%, these MNs are rapidly dissolved due to hydrolysis, which leads to a rapid deformation or disappearance of needles, and poor insertion ability into the skin [28,30].

Hyaluronic acid (HA), which is normally used as a common ingredient in skin care products, was found to produce MNs with high biocompatibility and resistance to deformation. The resulting MNs were strong enough to reliably pierce into skin, dissolve, and rapidly release the contained drug into the skin. Furthermore, the absence of a heating step and organic solvents during fabrication proved to be a notable advantage in preserving the stability of incorporated drugs, such as insulin. Therefore, in the present study, we developed novel insulin-loaded MNs fabricated from HA. Despite that previous researchers have already reported on the use of MNs fabricated from various materials in transdermal drug delivery, this is the first study to use and apply HA in the preparation of insulin-loaded MNs, although we have already reported that transdermal absorption of alendronate, a nitrogen-containing bisphosphonate was much improved using alendronate-loaded MNs fabricated from HA [32]. We also assessed the characteristics of MNs prepared from HA and their ability in transdermally delivering insulin to diabetic rats *in vivo*.

2. Materials and methods

2.1. Materials

HA was kindly provided by Shiseido Co., Ltd (Tokyo, Japan). Streptozotocin was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Pentobarbital sodium, citric acid buffer solution, and bovine insulin (28 U/mg) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Glucose CII and insulin-EIA test kits were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals and reagents were of analytical reagent grade.

Male Wistar rats, weighing 220 to 270 g, were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). All experiments were performed in accordance with the guidelines of the animal ethic committee at Kyoto Pharmaceutical University.

2.2. Fabrication of insulin-loaded MNs

MNs were fabricated via micromolding technologies, with HA as the base material, and then, were loaded with 0.13, 0.25, and 0.44 IU of bovine insulin. The fabrication process of MNs can be considered as transcription from the micromold with needle-shape in place. In detail, 15% HA solution was obtained by mixing well with distilled water. Insulin solution dissolved in 0.1 M HCl solution was added to the 15% HA solution and mixed well to prepare HA solution containing insulin. 0.3 ml of the resulting HA solution containing insulin was placed on a 2 cm × 2 cm micromold at room temperature. After 2 h drying in desiccator, the remaining solution was removed on the surface of mold with cotton, then 0.1 ml of 20% HA solution was placed on the same place of micromold. After drying the micromold completely, a 2 cm × 2 cm polyethylene terephthalate (PET) adhesive tape was attached on the baseplate for reinforcing. A sheet of insulin-loaded MNs was obtained by peeling the mold off. Insulin-loaded MNs in circular area with a diameter of 10 mm were obtained by cutting the sheet with a punch. The thickness of the MNs baseplate was 50 ± 5 μm. Recording to the fabrication process, the baseplate was considered to consist of HA and a small amount of insulin. We determined the amount of insulin in needles and baseplate, and more than 90% of insulin was detected in the needles. By cutting circular area of insulin loaded MNs from resulting sheet with a punch as shown in the process above, there was no sidewall formation in the resulting insulin loaded microneedles. Scanning electron microscopy was performed to examine the MNs.

We also determined the mechanical strength with a stress-strain gauge (FS1K, Imada, Japan) by pressing MNs against a stainless steel plate. When the top of MNs bended more than 45°, the force value was read from the meter. The failure force was calculated with the force divided by the area of MNs. The failure force of our MNs was 17.5 N/cm^2.

2.3. Hygroscopy of insulin-loaded MNs

Insulin-loaded MNs were stored at a high relative humidity of 75%. This condition was obtained by storing the MNs in a desiccator containing a saturated solution of sodium chloride. MNs were removed at pre-determined intervals, and the weight of MNs was measured and the percent change in MNs weight was calculated. No MN was returned into the container after testing.