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Two-layered dissolving microneedles formulated with intermediate-acting insulin

Yukako Ito[a,*], Muneyuki Hirono[a], Keizo Fukushima[b], Nobuyuki Sugioka[b], Kanji Takada[a,**]

[a] Department of Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan
[b] Department of Clinical Pharmacokinetics, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Hyogo, Japan

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ABSTRACT

Two-layered dissolving microneedles (DMs) containing intermediate-acting insulin, protamine sulfate insulin (PSI), were prepared. Then a pharmacodynamic study was performed to evaluate the prolonged hypoglycemic effects in rats. The DMs were approximately 497 ± 5 μm long, with 303 ± 3 μm diameter at their base. The length of the insulin loaded space was 182 ± 4 μm. PSI contents in DMs were 0.51 ± 0.02 IU. A three-month stability study showed that 99.9 ± 1.4% of PSI was recovered at 4 °C. As the temperature increased to 40 °C, recovery decreased to 97.5 ± 2.0%. PSI was released within 5 min from DMs. Hypoglycemic effects of PSI DMs were evaluated in rats where subcutaneous injection preparations were used as references. Total area above the plasma glucose level (% of the pre-dose level) vs. time curve as an index of hypoglycemic effect was 144.0 ± 16.0% h and 243.3 ± 8.5% h for PSI DMs at 1.46 and 3.28 IU/kg. The relative pharmacologic availability of PSI from DMs were 100.2 ± 9.8% and 91.4 ± 4.1%. No significant difference of hypoglycemic curves was found between DMs and injection solutions, which suggests the usefulness of two-layered DMs of PSI for the displacement therapy of sc injection preparation.

1. Introduction

Dissolving microneedles (DMs) are useful transdermal drug delivery system (TDDS) for poorly permeable drugs, of which insulin is a representative drug (Ito et al., submitted for publication, 2006a, 2008a). The DMs are made of a water-soluble thread-forming biopolymer such as chondroitin sulfate, dextran, hyaluronic acid, and albumin, which can be used as the base polymer (Ito et al., 2008b,c,d, 2006b; Takada, 2008). For DM, drugs are formulated at the acral portion of microneedles as a solid dispersion or suspension with base polymer. The remaining portions of the needles are formed with the base polymer itself. From the standpoint of manufacturing processes, 200–300 two-layered DMs are formed as an array on a chip having the diameter of 17.0 mm (Takada, 2008). DMs have an advantage over other types of microneedles, surface coated microneedles, hollow-type microneedles, and pierce type microneedles. Those microneedles are not pharmaceuticals but medical devices. Surface coated microneedles were applied to skin vaccine where small amounts of antigen, approximately 10 μg, were formulated (Matriano et al., 2002; Zhu et al., 2009). Laser-engineered dissolving microneedle arrays has a problem of toxicity derived from polymer component (Migalska et al., 2011). Hollow type microneedles require a reservoir into which the drug solution is filled (Roxhed et al., 2008; Gupta et al., 2009). Peptide/protein drugs often have a problem of stability. Especially in the case of injection preparation, the drug solution must be stored at low temperature, i.e., in a refrigerator. Pierce type microneedles merely have the function of making micropores in the skin. After micropores are formed, a sponge containing a drug solution or cream is applied onto the skin. With such a system, Wermeling et al. (2008) performed a clinical phase I study and showed that the appearance of a skin-impermeant drug, naltrexone, into the systemic circulation after micropores were made by insertion of a stainless-steel made microneedle sheet onto the human skin, where naltrexone was applied using a patch preparation. In their studies, plasma drug concentrations were measured, providing evidence that naltrexone were absorbed from the skin into the systemic circulation.

In contrast, DM is a pharmaceutical preparation whereby drug molecules are delivered into the skin merely by pushing DM onto the skin with a finger. Our previous study showed that the dissolution of DM was completed within 5 min. Then, FITC-insulin was released rapidly both horizontally and vertically in the skin (Fukushima et al., 2010). A pharmacodynamic study of insulin DMs showed no significant difference for the minimum plasma glucose level, C_{min}, and T_{min} when C_{min} appeared in rats between DM

* Corresponding author. Tel.: +81 75 595 4626; fax: +81 75 595 6311.
** Corresponding author.
E-mail addresses: yukako@mb.kyoto-phu.ac.jp (Y. Ito), takada@mb.kyoto-phu.ac.jp (K. Takada).

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preparation and subcutaneous (sc) injection of insulin solution (Ito et al., submitted for publication). A safety study showed no irritation on the rat skin after administration of insulin DM (Ito et al., submitted for publication; Fukushima et al., 2010). With DM containing native insulin, the duration of the hypoglycemic effect in rats was 3 h, which was almost equivalent to that obtained after sc injection of insulin solution. Clinically, insulin preparations of several kinds are used, short-acting, intermediate-acting, and long-acting ones. Through our research, it has been confirmed that insulin DM had a short onset time in the hypoglycemic effect after administration to the skin. As the next step, we want to ascertain whether DM is applicable for intermediate-acting insulin. The mechanism by which protamine sulphate inhibits complement activation in vitro could be related to its ability to interfere with the physical nature of the solid surfaces presented by the insulin crystals (Duchateau et al., 1992). Therefore, two-layered DMs containing intermediate-acting insulin, protamine sulfate insulin (PSI), were prepared and then evaluated using a pharmacodynamic study in rats.

2. Materials and methods

2.1. Materials

Protamine sulfate insulin (PSI) was obtained from Novolin N™ (Novo Nordisk Japan, Tokyo, Japan) by centrifugation for 10 min and drying in the oven for one day at 50 °C. A Glucose CII-Test kit, brilliant blue (BB), and sodium chondroitin sulfate were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Mercodia insulin ELISA assay kit was obtained from Funakoshi Co. Ltd. (Tokyo, Japan). Male Wistar Hannover rats used in the study were obtained from Japan SLC, Inc. (Hamamatsu, Japan). A standard solid-meal commercial food (LabDiet™; Nippon Nousan Ltd., Yokohama, Japan) was used. All other materials were of reagent grade and were used as received.

2.2. Preparation of DM chip

First, the BB solution was prepared by dissolving 1.0 mg of BB with 3 ml of the supernate obtained after centrifugation of Novolin N preparation. To the mixture of PSI 5.0 mg, chondroitin sulfate 15.0 mg, and protamine sulfate 10.0 mg, 60 μl of BB solution was added. By knedding, hydrogel glue where PSI was suspended was obtained. After the obtained hydrogel glue was degassed under reduced pressure, it was dispensed into a mold containing 225 inverted cone-shaped wells with area of 1.0 cm^2 by squeegee technique. Each well had 500 μm depth and 300 μm diameter at its top. The mold was covered with a 300 g steel plate to prevent the squirt of the hydrogel and the drug glue was dried being filled into the wells. After the plate was removed, glue consisting of 15 mg of chondroitin sulfate and 25 ml of distilled water was painted over the mold and dried under the pressure of a stainless steel plate for 3 h. Thereafter, the plate was removed and DMs were obtained by detaching them with a supporting material. Placebo DMs were prepared using the same method, but PSI was not added to them.

2.3. Microscopic observation of DMs

A PSI DM array chip of which the acral portion was stained with BB was observed using a digital videomicroscope (VH-5500; Keyence Co., Osaka, Japan) under normal light.

2.4. PSI contents in DMs

PSI was extracted from a chip having DMs with 5.0 ml of phosphate buffer, pH 7.4, for 10 min. The obtained extract was centrifuged at 13,000 rpm for 5 min at 8 °C using a centrifuge (Kubota 3700; Kubota Corp., Tokyo, Japan). The supernate was separated from the debris and was stored in a deep freezer at −80 °C until analysis.

2.5. HPLC assay method of PSI

Onto an HPLC system (LC-10A; Shimadzu Corp., Kyoto, Japan) equipped with a UV detector (SPD-10A; Shimadzu Corp., Kyoto, Japan) and a Nucleosil 5C18 ODS column, 4.6 mm × 150 mm (Chemco Scientific Co. Ltd., Osaka, Japan), 100 μl of the PSI sample solution was injected. The mobile phase consisted of 0.2 M anhydrous sodium sulfate adjusted to pH 2.3 using *ortho* phosphoric acid and acetonitrile (74:26, v/v). The flow rate was 1.5 ml/min; the column temperature was 36 °C. The detection wavelength of PSI was 214 nm, as described in a previous report (Sheshala et al., 2007).

2.6. In vitro dissolution experiment

In vitro dissolution experiments were performed using a DM array chip with 3.0 ml of PBS, pH 7.4, as the dissolution medium at 37 °C. To determine the amount of PSI released from DM array chip, 0.2 ml of the dissolution medium was collected for the assay for 30 min at the predetermined time, 0, 0.25, 0.5, 0.75, 1.0, 1.5 2.0, 3.0, 5.0, 10.0, 20.0, and 30.0 min and thereafter replaced with fresh dissolution medium that had been degassed earlier. The cumulative released amount of PSI from DM array chip was defined by the following equation.

$$\text{Cumulative amount released} = \left(\sum_{t=0}^{t} \frac{M_t}{M_{30\ \text{min}}} \right) \times 100\%$$

therein, M_t is the amount of PSI dissolved at time t, and $M_{30\,\text{min}}$ is the dissolved amount of PSI at 30 min after the start of the dissolution experiment.

2.7. Stability experiment

DM array tips were kept under four different conditions, i.e., −80, 4, 23, and 40 °C for 1 and 3 months. Thereafter, DM array chips were dissolved with 1.0 ml of phosphate buffered saline (PBS) of pH 7.4. Then the PSI contents were measured using HPLC method.

2.8. In vivo absorption experiments in rats

Male Wistar Hannover rats, 331 ± 17 g, were anesthetized with an intraperitoneal injection of sodium pentobarbital, 50 mg/kg. One group consisted of 3–4 rats. At 5 min before drug administration, 0.25 ml of blank blood samples was obtained from the left jugular vein with a heparinized syringe. The hair on the abdominal region was removed with a shaver. The DMs were administered to the skin by pressing with fingers and pressed for 3.0 min. There was no bleeding after administration. At 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, and 8.0 h after administration, 0.2 ml of blood samples was collected from the left jugular vein. By centrifuging at 12,000 rpm for 10 min at 4 °C using a centrifuge (Kubota 1700; Kubota Corp., Tokyo, Japan), 100 μl plasma samples were obtained. The resultant plasma samples were stored at −80 °C until analysis.

For the sc injection experiment, PSI solution was injected to rats after ten times dilution, 1.50 and 3.5 IU/kg. After a 0.25-ml blank blood sample was obtained from the left jugular vein, additional blood samples of 0.25 ml were obtained at the same sampling schedule using a heparinized syringe. After centrifugation, plasma samples were obtained. All these plasma samples were frozen immediately in a deep freezer at −80 °C until analysis.