Self-Dissolving Micropiles for the Percutaneous Absorption of Recombinant Human Growth Hormone in Rats

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The feasibility of self-dissolving micropiles (SDMP) as a percutaneous delivery system of recombinant human growth hormone (rhGH) has been studied in rats using SDMP where dextran was used as a base. After mixing dextran solution with rhGH, SDMPs were prepared by pulling with polypropylene tips. The mean weight, length and diameter were 0.68±0.05 mg, 3.2±0.5 mm and 0.6±0.2 μm, respectively. To evaluate the bioavailability (BA) of rhGH percutaneously administered by SDMP, an absorption experiment was performed in rats. RhGH SDMPs were inserted into the rats skin, 200 μg kg⁻¹, and plasma rhGH levels were measured by an ELISA method. Peak plasma rhGH level, 132.8±11.8 ng ml⁻¹, appeared at 0.8±0.2 h. By comparing the plasma rhGH levels vs. time profiles after the administration of SDMP and intravenous injection of rhGH solution, 5 μg kg⁻¹, BA of rhGH from SDMP was calculated to be 87.5%. These results may suggest that SDMP can be used as a novel percutaneous drug delivery system.

Key words recombinant human growth hormone; micropile; percutaneous administration; absorption; rat

Growth hormone (GH) is anabolic, stimulates muscle development and strength, enhances the utilization of fat, and increases bone mineral density. These effects are exerted directly by GH and via its stimulation of insulin-like growth factor I production. Recombinant human growth hormone (rhGH) has been used as a therapy for growth hormone deficieny (GHD) in children. For adult patients with burns, bone fractures and Turner’s syndrome, rhGH is also effective. The effects of rhGH on adult GHD are dyslipidemia, greater bone mass and better quality of life (QOL). Presently, rhGH is administered as a subcutaneous injection daily or three times a week. However, the QOL of patients is low. Although a pen device fitted with a fine needle was developed, some patients, especially children, fear injections and noncompliance often becomes a problem in a clinical situation. For children, needle-free jet injector has been developed. To increase the adhesiveness of rhGH to patients to another delivery system would be clinically valuable. Many attempts have been made to develop a new dosage form of rhGH: intranasal delivery, oral delivery, oral liposome preparation and microcapsules. However, none of them has succeeded as a new rhGH delivery system.

A transdermal therapeutic system is a useful approach to administering rhGH; however, the barrier function of the skin is strong. To increase the skin permeability, many different approaches have been studied including chemical/lipid absorption enhancers, electroporation, ultrasound and thermal methods. The success of these transdermal drug delivery systems however has been limited because of low membrane permeability of drugs through the skin. Therefore, we designed self-dissolving micropiles (SDMP) for the percutaneous administration of insulin and erythropoietin and a pharmacological availability of insulin of over 91% was obtained in mice.

In this report, SDMP was utilized with rhGH and the absorption efficiency of rhGH from SDMP was studied.

MATERIALS AND METHODS

Materials RhGH was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dextran (MW=50000—70000) was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Male Wistar rats used in the study fed a standard meal of commercial food (LabDiet®) were obtained from Japan SLC Inc. (Hamamatsu, Japan). All other materials were of reagent grade and were used as received.

Preparation of rhGH SDMP RhGH, 1.4 mg, was dissolved with 80 μl of deionized distilled water. To this solution, 40.2 mg of dextran was added and glue was obtained by mixing well. The mixture was pulled with the aid of polypropylene tips and piles were formed. After the tips to which thread was attached had been dried in a desiccator, micropiles were obtained. The mean weight of the SDMPs was 0.68±0.05 mg. Three SDMPs were administered to one rat. RhGH solution for intravenous (i.v.) injection study was prepared by dissolving 0.25 mg of rhGH with 50 ml of saline of which 0.2 ml was i.v. injected to rats, 5 μg kg⁻¹.

In Vivo Absorption Experiments Male Wistar rats, 343±31 g, were anesthetized with an intraperitoneal injection of sodium pentobarbital, 50 mg kg⁻¹. One group was composed of 3 rats. Five minutes before drug administration, a blank blood sample, 200 μl, was obtained from the left jugular vein. After the hair of the abdominal skin was removed, rhGH SDMP was percutaneously inserted into the epidermis. In administering SDMP to the rat skin, the site of administration was not the dermis but the epidermis as confirmed by there being no hemorrhage. Five, 10, 20, 30 minutes and 1, 2, 4, 6, 8 h after administration, blood samples were obtained from the left jugular vein with a heparinized syringe. For i.v. injection study, rhGH solution, 5 μg ml⁻¹, was prepared by dissolving rhGH in saline and was injected into the right jugular vein, 5 μg kg⁻¹. After blank blood samples were obtained from this vein, 0.1 ml samples were obtained at 2, 5, 10, 30 min and 1, 2 h with a heparinized syringe. By centrifuging at 12000 rpm for 10 min, 100 μl of the plasma samples were obtained. All these plasma samples were immediately frozen in a deep freezer at −80 °C until analyzed. All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation, Kyoto Pharmaceutical University.

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**Plasma rhGH Assay** The plasma rhGH levels were measured by enzyme-linked immunosorbent assay (ELISA) method using an Immunoassay Kit (BioSource International, Inc., California, U.S.A.). The method is a solid phase enzyme amplified sensitivity immunoassay performed on a microtiter plate. The rhGH concentration of the plasma samples was determined against a standard curve of rhGH in rat plasma. The variability of the assay was evaluated using the data obtained for rhGH quality control samples prepared in rat plasma and the standard curve. The validation parameters of precision (CV less than 15%) and accuracy (recovery of ±20%) were acceptable and the lower limit of quantitation was 1.0 ng ml⁻¹. The basal endogenous rhGH levels were measured as zero in all the animals. The plate-shaker and plate-washer used were a Titramax 101 (Heidolph Instruments, Germany) and a Dia-washer II (Dia-Iatron Co., Ltd., U.S.A.), respectively. Absorbance was measured at 450 nm using a MTP-300 microplate reader, (Corona Electric, Japan).

**Pharmacokinetic Analysis** Pharmacokinetic parameters were determined from the plasma rhGH concentrations vs. time data by a noncompartmental pharmacokinetic analysis method using WinHARMONY software we developed.²⁹ The maximum drug concentration (C_max) and the time to reach maximum concentration (T_max) were determined from the actual plasma concentration–time data. The area under the plasma drug concentration vs. time curve for 0—8 h (AUC) after percutaneous administration was calculated using the linear trapezoidal rule up to the last measured drug concentration and the percent BA was calculated by the following equation:

\[
\%BA = \left( \frac{AUC_{\text{percutaneous}}}{AUC_{i.v.}} \right) \times \left( \frac{\text{Dose}_{i.v.}}{\text{Dose}_{\text{percutaneous}}} \right) \times 100
\]

**Statistics** All values are expressed as their mean±S.E. Statistical differences were assumed to be reproducible when \( p<0.05 \) (Student’s unpaired t-test).

**RESULTS AND DISCUSSION**

SDMP were administered to the rat skin by insertion into the abdominal skin where the dose of rhGH was 200 μg kg⁻¹. Figure 1 shows the plasma rhGH concentration vs. time curves after administration. Plasma rhGH level rapidly increased and reached its maximum level within about 1 h. The mean maximum concentration, C_max, was 132.8±11.8 ng ml⁻¹. Thereafter, plasma rhGH level gradually decreased and declined to 11.7±2.4 ng ml⁻¹ at 8 h. On the other hand, rhGH solution was i.v. injected to another group of rats and the plasma rhGH concentration vs. time curves are also shown in Fig. 1. Just after i.v. injection of rhGH, mean plasma rhGH level, 94.8±20.7 ng ml⁻¹, was obtained and its level rapidly decreased within 2 h. Pharmacokinetic analysis based on noncompartmental analysis was performed with those data and the results are shown in Table 1. The AUCs of rhGH obtained after percutaneous and i.v. administrations were 12.4±2.1 and 432.9±25.3 ng·h ml⁻¹, respectively. As the dose differed, bioavailability of rhGH was calculated by comparing the two AUCs corrected by the administered dose. The absolute BA of rhGH from SDMP was 87.5%, which was not significantly different from i.v. administrations. The orally active growth hormone secretagogue, SM-130686, was determined and plasma rhGH level responded to this secretagogue after oral administration of a high dose of 10 mg kg⁻¹ to rats. However, the C_max was about 75 ng ml⁻¹ and T_max was 20 min.²⁰ Therefore, the clinical need for rhGH is still high. To increase the QOL of pediatric dwarfism patients, dosage forms other than the injection preparation have been studied, and nasal and oral drug delivery systems have been attempted.²⁷—²⁹ Cheng et al. designed an intranasal chitosan/rhGH formulation and BA was evaluated in sheep, though the BA was around 15%. The bioavailability of rhGH of oral formulation is usually lower than nasal absorption, though the BA value was not shown.²⁸,²⁹ The clinical dose of rhGH for subcutaneous (s.c.) injection is 6—12 μg kg⁻¹ in adults when used as therapy for GH deficiency. Assuming the body weight of a 60 kg adult, 360—720 μg of rhGH is required.³¹ However, if the BA is 15%, 2.4—4.8 mg of rhGH is required for nasal administration. To obtain higher bioavailability of rhGH, SDMP was used in this study, and since high BA of 87.5% was obtained, about 400—800 μg of rhGH can be used for human patients. This is a great advantage of SDMP over other non-injectable rhGH DDSs. It is also reported that endogenous GH has a circadian rhythm, namely much more GH is secreted at night than during the day. In addition, GH is secreted approximately every 3 h by the pulse.²² In the s.c. injection of rhGH, it is difficult to divide the daily rhGH dose into 2 or 3 injections. SDMP is a painless DDS for the percutaneous administration of rhGH. Therefore, rhGH can be administered to patients several times a day, especially just before sleep, and QOL of patients will increase.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Administration route</th>
<th>Dose (μg kg⁻¹)</th>
<th>C_max (ng ml⁻¹)</th>
<th>T_max (h)</th>
<th>AUC₀⁻₈ (ng·h ml⁻¹)</th>
<th>BA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>i.v.</td>
<td>5</td>
<td>94.8±20.7</td>
<td>—</td>
<td>12.4±2.1</td>
<td>100</td>
</tr>
<tr>
<td>SDMP</td>
<td>Percutaneous</td>
<td>200</td>
<td>132±11.8</td>
<td>0.8±0.2</td>
<td>432.9±25.3</td>
<td>87.5</td>
</tr>
</tbody>
</table>

C_max: peak plasma rhGH concentration, AUC: area under plasma rhGH concentration vs. time curve, BA: bioavailability. Each point shows the mean±S.E. of 3 experiments.

![Plasma rhGH Concentration vs. Time Curves after i.v. Injection of Solution and Percutaneous Administration of SDMP to Rats](image)
Microfabrication technology has advanced in mechatronics fields, where silicone or metal is used for fabrication. However, it is difficult to use these materials as a pharmaceutical base because of the safety problem. Our SDMP includes biodegradable polymers like dextran, dextrin and chondroitin sulfate, which were used in our previous studies on mice and rats.\textsuperscript{23—25) Those polymers are rich in the human body. Therefore, safety is no problem for the clinical use of these polymers. Indeed, a safety study was performed in our experiment. After the administration of SDMP to rat abdominal skin, a histological examination was made, and no damage was detected on the skin. Therefore, we are able to state that rhGH SDMP will be a good novel dosage form for the percutaneous delivery of rhGH.

CONCLUSIONS

The possibility using SDMPs for the administration of rhGH has been studied in rats. SDMP containing rhGH was administered 200\,\mu g\,kg\(^{-1}\) of the rat skin. By comparing the \(\text{AUC}\) of SDMP and that obtained after i.v. injection of 5\,\mu g\,kg\(^{-1}\) of rhGH solution, BA of rhGH from SDMP was calculated to be 87.5\%. The possibility of SDMP as a useful delivery system for percutaneous absorption of rhGH was recognized.

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