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Evaluation of self-dissolving needles containing low molecular weight heparin (LMWH) in rats

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Abstract

Feasibility study of self-dissolving needles containing polysaccharide was performed. Low molecular weight heparin (LMWH) was used as a representative polysaccharide. Using chondroitin, dextran and dextrin as the base, self-dissolving needles (SDN) were prepared. The obtained SDNs were evaluated in rat absorption experiment, where pharmacological availability (PA) was calculated by comparing the plasma anti-Xa activity *vs*. time curves between SDNs and i.v. solution. After the insertion of SDNs to rats skin where the doses of LMWH were 25, 50 and 100 IU/kg, plasma samples were collected for 6 h and anti-Xa activity was measured as the pharmacological index of LMWH. The anti-Xa level was maintained above 0.2 IU/ml, the therapeutic level, for about 2 h at a dose of 100 IU/kg. Almost the same PAs of LMWH were obtained with dextran and dextrin SDNs, 97.7% and 102.3%, though lower PA was obtained with chondroitin SDN, 81.5%. *In vitro* dissolution experiment showed that LMWH was released from dextran, dextrin and chondroitin SDNs within 10 min. The $T_{50\%}$s were 0.84 ± 0.06 min for dextran SDN, 1.07 ± 0.12 min for chondroitin SDN and 2.11 ± 0.31 min for dextrin SDN, respectively. Plasma anti-Xa activity *vs*. time profiles showed good dose-dependency in the 25–100 IU/kg range and high PAs were obtained, 90.0% for 25 IU/kg, 95.4% for 50 IU/kg and 97.7% for 100 IU/kg from dextran SDNs. Stability experiment was performed with dextran SDNs for 3 months. Above 97% of LMWH were remained in SDNs under three different conditions, −80, 4 and 40 °C. These results suggest the usefulness of SDN to polysaccharide drug.

Keywords: Low molecular weight heparin; Self-dissolving needles; Percutaneous administration; Pharmacological availability; Anticoagulant; Rats

1. Introduction

Transdermal drug delivery system (TDDS) made a great progress of DDS and several drugs like estrogen, nicotine and nitroglycerin are clinically supplied as patch preparations (Asmussen, 1991). However, the skin has a strong barrier function for the permeation of other drugs, especially macromolecular drugs. To increase the skin permeability, many approaches including chemical enhancers, electric fields, ultrasound and thermal methods have been challenged (Barry and Williams, 2003; Cevc, 2004; Preat and Vanbever, 2004; Doukas, 2004; Mitragotri and Kost, 2004). However, the application of these TDDSs has been limited because of the strong barrier function of the skin. To overcome these problems, microneedles are under investigation actively (Cormier et al., 2004; Martanto et al., 2004; Davis et al., 2005; Park et al., 2006). We have been studying a new delivery system, self-dissolving microneedles, for the percutaneous administration of macromolecular drugs. In our feasibility studies, insulin and erythropoietin (EPO) were used as representatives of peptide/protein drugs (Ito et al., 2006a,b). With insulin, the efficiency was evaluated by measuring the hypoglycemic effect of insulin after administration of insulin self-dissolving microneedles to mice. The pharmacological availability (PA) of SDN was over 90% as compared to intravenously injected insulin solution (Ito et al., 2006a). Also, EPO self-dissolving microneedles showed high bioavailability (BA) of EPO after percutaneous administration to mice where the BA was about 80% (Ito et al., 2006b). Through these proof-of-concept experiments, the usefulness of self-dissolving

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microneedles was found out with peptide/protein drugs. On the other hand, there are many macromolecular drugs except peptide/proteins. One of the categories is polysaccharide like heparin.

Heparin is an anticoagulant drug for the treatment of deep vein thrombosis and pulmonary embolism (Agnelli and Sonaglia, 2000; Ageno, 2000). Unfractionated heparin (UH) is a naturally occurring glycosaminoglycan that exists as a heterogenous mixture of oligosaccharides composed of alternating chains of D-glucosamine and uronic acid (Hirsh et al., 1992). Heparin is sulfated, highly acidic and has a negative ionic charge. In many countries, low molecular weight heparins (LMWHs) have replaced UH for the prevention and treatment of venous thrombo-embolism (Boneu, 2000) due mainly to a longer half-life and less bleeding for a given antithrombotic effect compared to UH. Furthermore, the frequency of heparin-induced thrombocytopenia is lower with LMWHs and almost non-existent when used in the short-term because of less immunogenic nature (Lane et al., 1984). LMWHs have a molecular weight of approximately 4.5 kDa and, compared to UH (12.0 kDa), show better distribution and less binding to non-anticoagulant-related plasma proteins and platelets (Heit, 1998). However, LMWH is not absorbed from the gastrointestinal tract owing to its high charge density and large molecular size and is only given by injection.

Therefore, LMWH was used as a representative polysaccharide drug and feasibility study of self-dissolving needles (SDN) for the percutaneous administration of LMWH has been studied in this report.

2. Materials and methods

2.1. Materials

LMWH (Parnaparin sodium, anti-Xa (aXa) factor activity: 85.4 IU/mg) was obtained from Ajinomoto Co. Ltd. (Tokyo, Japan). Dextrin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Chondroitin sulfate and dextran was obtained from Nacalai Tesque Co. Ltd. (Kyoto, Japan). Male Wistar rats used in the study were obtained from Nippon SLC Company (Hamamatsu, Japan) and standard solid meal of commercial food (LabDiet®) was purchased from Nippon Nousan Co., Ltd. (Yokohama, Japan). All other materials used were of reagent grade and were used as received.

2.2. Preparation of LMWH SDN

Twenty milligram of LMWH was dissolved in 20 μl of deionized water. To 40 mg dextrin or chondroitin sulfate or dextran, 20 μl of LMWH solution was added and glue was obtained by mixing well. The mixture was spread with the aid of polypropyrene tips and long thread-like needles were formed. After the tips to which thread is attached was dried in a desiccator, SDNs were obtained. Before administration, SDNs was cut to adjust the weight of SDNs to rats' body weight. The mean weight of the SDN was 1.24 ± 0.01 mg for an experiment at a dose of 100 IU/kg. The mean length and basal diameter were 1.52 ± 0.12 and 0.51 ± 0.02 mm, respectively (Fig. 1(a)). One SDN was administered to one rat, as shown in Fig. 1(b).

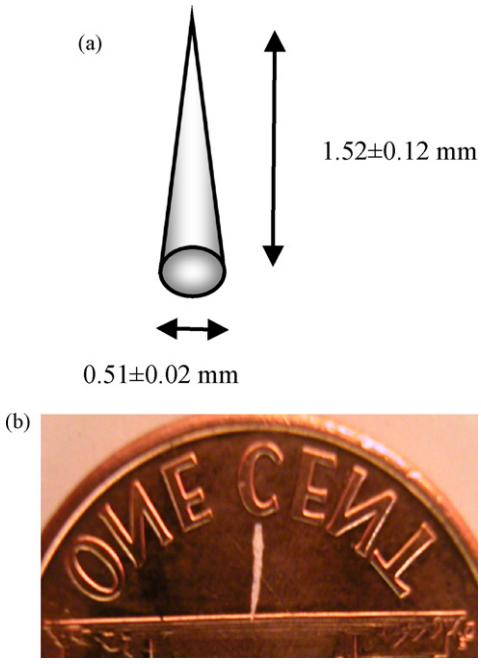

Fig. 1. (a) Schematic representation of the used self-dissolving needle (SDN). (b) Microscopic photograph of SDN on a cent coin.

2.3. In vivo absorption experiments with rats

Male Wistar rats (320–350 g) were used after fasted overnight for at least 12 h were used in the study. The animals were anaesthetized by intraperitoneal administration of sodium pentobarbital solution (50 mg/kg). One group composed of three to four rats. At 5 min before administration, blank blood sample, 0.1 ml, was obtained from the left jugular vein. After the hair of the abdominal skin was removed, the LMWH SDN was percutaneously inserted into the skin with device. When SDN was administered to the rat skin, applicator was used. We confirmed that SDN was inserted into the rat skin by a direct observation with an eye. As one SDN was inserted into the skin of each rat, it was easy to identify the SDN in the skin. At 0.5, 1, 2, 3, 4, 5 and 6 h after the administration. Blood sample, 0.1 ml, was collected from the left jugular vein into syringes containing 0.0l ml of 3.2% (w/v) trisodium citrate dihydrate solution as anticoagulant. The samples were mixed well and immediately cooled on an ice bath. Plasma was obtained from whole blood by centrifugation at 5000 rpm for 15 min at 4 °C using Kubota 1720 centrifuge (Tokyo, Japan), and then stored at −80 °C until analysis. Factor Xa inhibition activity in plasma samples was measured by the Hemos IL™ (Instrumentation Laboratory, Italy). For i.v. injection study, LMWH solution was prepared by dissolving LMWH in saline (pH 7.4) and was injected into the right jugular vein, 40 IU/kg, after blank blood samples were obtained from the left jugular vein. Blood samples, 0.1 ml, were collected at 1, 5, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min. Plasma fraction was obtained and stored as noted above and anti-Xa ativity was measured. All animal experiments were

all carried out in accordance with the Guidelines for Animal Experimentation, Kyoto Pharmaceutical University.

2.4. In vitro dissolution experiments

SDN, 0.75 mg, were put into a small bag made of tissue paper and the resultant bag was kept in 100 ml of phosphate buffer, pH 7.4. The dissolution medium was degassed by sonication at room temperature and maintained at 37 °C throughout the test period. Dissolution tests of the LMWH-loaded SDPMs were carried out according to JP XIII method (paddle method) in reduced scale. The paddle was used with a rotation speed of 100 rpm. To determine the release of LMWH from SDNs, 0.2 ml samples of the dissolution medium were removed for analysis at 0, 10, 20, 30, 40, 50 s and 1, 1.5, 2, 3, 5, 10, 15, 30 min. Samples were subsequently replaced with fresh dissolution medium. Factor Xa inhibition activity in the dissolution samples was measured by the Hemos IL^{TM} (Instrumentation Laboratory, Italy). The value of $T_{50\%}$, the time when half of the formulated amount of LMWH was released from the SDNs, was determined by an interpolation method.

2.5. Stability experiment

SDNs, 0.75 mg, were kept under three different conditions, i.e., −80, 4 and 40 °C for 1 and 3 months. Thereafter, SDNs were dissolved with 100 ml of saline, pH 7.4, and LMWH contents were measured by the Hemos IL^{TM} (Instrumentation Laboratory, Italy).

2.6. Pharmacokinetic analysis

The time to reach maximum plasma anti-Xa activity (T_{max}) and the maximum plasma anti-Xa activity (C_{max}) were determined from the authentic plasma anti-Xa activity *vs*. time data. The area under the plasma anti-Xa activity *vs*. time curve up to infinity ($AUA_{0-\infty}$) after administration of the test preparations were calculated using the multi-line fitting. The bioavailability (BA) was calculated by using the following equation.

$$\%BA = \left(\frac{AUA_{SDN}}{AUA_{i.v.}}\right) \times \left(\frac{DOSE_{i.v.}}{DOSE_{SDN}}\right) \times 100$$

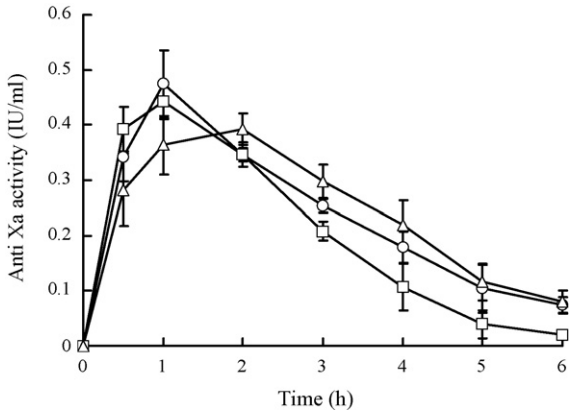

Fig. 2. Effect of base polymers on plasma anti-Xa activity-time profiles after percutaneous administrations of LMWH SDNs to rats, 100 IU/kg. (◯) Dextran; (□) chondroitin; (△) dextrin. Each point shows the mean ± S.E. of three experiments.

2.7. Statistical analysis

All values are expressed as their mean ± S.E. Means of two groups were compared using non-paired Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

3. Results

LMWH loaded SDN was evaluated in the rat absorption experiment and the results are shown in Fig. 2. Self-dissolving needle is composed of drug and polymer base. After SDN was inserted into the skin, both drug and polymer dissolved with the environmental water and thereafter the drug was thought to be absorbed into the systemic circulation. At first, the effect of base polymer on the absorption of LMWH from rat skin was studied. Dextran and chondroitin based SDNs showed almost the same plasma anti-Xa activity *vs*. time profiles. However, dextrin SDN had slower absorption rate of LMWH from the preparation. Table 1 shows the pharmacokinetic parameters of LMWH from the three kinds of SDN preparations. The plasma anti-Xa activity levels, C_{max}s, were almost the same levels in the three SDNs, though T_{max} (when plasma anti-Xa activity shows its maximum level), 1.67 ± 0.33 h, of dextrin SDN was significantly different from chondroitin SDN, 0.83 ± 0.17 h ($p < 0.05$). The absorption rate of LMWH from dextrin SDN was slower than that from dextran and chondroitin SDNs. To determine the

Table 1
Pharmacokinetic parameters of anti-Xa activity after percutaneous administrations of SDNs and i.v. injection of LMWH solution to rats

Dosage form	Base	Dose (IU/kg)	C_{max} (IU/ml)	T_{max} (h)	$AUA_{0-\infty}$ (IU h/ml)	BA (%)
i.v. Solution	–	40	–	–	0.63 ± 0.03	100
SDNs	Dextran	100	0.47 ± 0.06	1.00	1.49 ± 0.08	97.7
SDNs	Dextran	50	0.25 ± 0.03	1.17 ± 0.4	0.73 ± 0.14	95.4
SDNs	Dextran	25	0.14 ± 0.02	1.00	0.35 ± 0.08	90.0
SDNs	Chondroitin	100	0.46 ± 0.03	0.83 ± 0.17	1.24 ± 0.10	81.5
SDNs	Dextrin	100	0.40 ± 0.03	1.67 ± 0.33[a]	1.56 ± 0.23	102.3

Each value represents the mean ± S.E. three experiments.
[a] Significantly different from chondroitin sulfate Na.

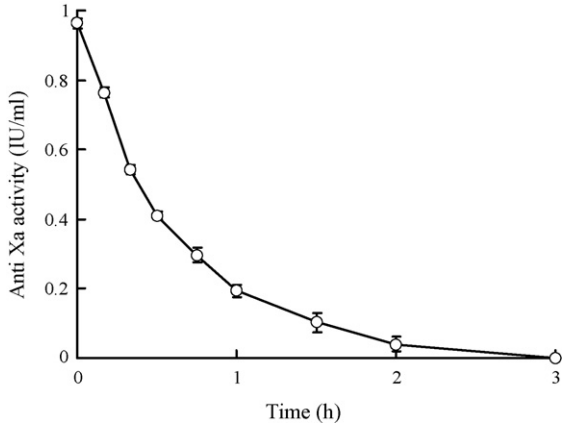

Fig. 3. Plasma anti-Xa activity-time profiles after intraveneous administrations of LMWH solution to rats, 40 IU/kg. Each point shows the mean ± S.E. of three experiments.

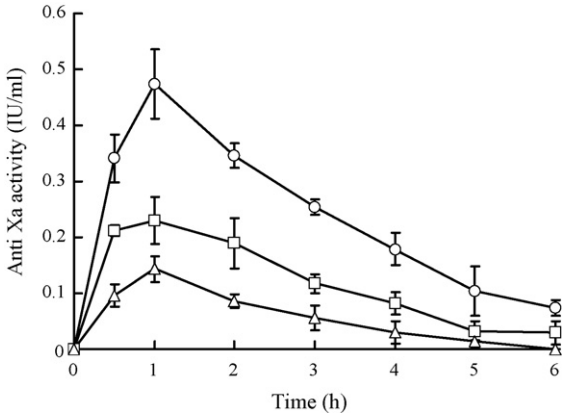

Fig. 5. Plasma anti-Xa activity-time profiles after percutaneous administration of dextran SDNs to rats. (△) 25 IU/kg; (□) 50 IU/kg; (◯) 100 IU/kg. Each point shows the mean ± S.E. of three experiments.

physiological availability (PA) of LMWH from SDNs, LMWH solution was intravenously (i.v.) injected to other group of rats at the LMWH dose of 40 IU/kg and Fig. 3 shows the plasma anti-Xa activity *vs*. time profiles. Just after the i.v. injection of LMWH solution, plasma anti-Xa activity showed the maximum level, 0.96 ± 0.01 IU/ml, and activity disappeared from the plasma within 3 h after i.v. injection. When LMWH was administered to rats by SDN, absorption process takes long time because LMWH has low membrane permeability. The mean residence time of LMWH after administration by SDN is longer than that obtained by i.v. injection. Therefore, the blood sampling time differs between the two experiments. By comparing the AUAs of these data, the PA of LMWH were calculated to be 97.7% for dextran SDN, 81.5% for chondroitin SDN and 102.3% for dextrin SDN, respectively.

To study the difference on the absorption rate of LMWH from the test SDNs, *in vitro* release experiment was performed and the results are shown in Fig. 4. After the start of the experiment, LMWH was released from SDNs very fast and almost all of the formulated LMWH was released within 10 min. The $T_{50\%}$ was estimated to be 0.84 ± 0.06 min for dextran SDN, 1.07 ± 0.12 min for chondroitin SDN and 2.11 ± 0.31 min for dextrin SDN, respectively. $T_{50\%}$ of dextrin SDN was significantly different from dextran SDN and chondroitin SDN ($p < 0.05$).

The effect of LMWH dose on the plasma anti-Xa activity *vs*. time profile after the percutaneous administration of the dextran SDNs was studied and the result is shown in Fig. 5. By increasing the LMWH dose from 25 to 50 and 100 IU/kg, C_{max} increased with proportional to the administered LMWH dose, from 0.14 to 0.25 and 0.47 IU/ml. The pharmacokinetic parameters are summarized in Table 1. AUA also increased from 0.35 to 0.73 and 1.49 IU h/ml in correlated to the dose and there were no significant differences on the PAs between three kinds of SDNs. Fig. 6 shows the dose-dependencies on C_{max} and AUA. There are good correlations between C_{max} or AUA and LMWH dose, $r = 0.99$ for C_{max} and $r = 1.00$ for AUA. Sugiyama et al. (1992) reported that plasma anti-Xa activity *vs*. time profile showed dose-dependency till the dose of 25 mg/kg, i.e., 2000 IU/kg. Their range of plasma anti-Xa-activity levels was 10–0.001 IU/ml. They stated that LMWH showed a linear kinetics, though the dose used in their study was extremely high dose as compared to the clinical situation, dose was from 5 to 25 mg/kg. The plasma anti-Xa activity obtained in our rat experiment was 0–0.5 IU/ml. We confirmed that the dose used in our study was within the range of linear kinetics of LMWH. There-

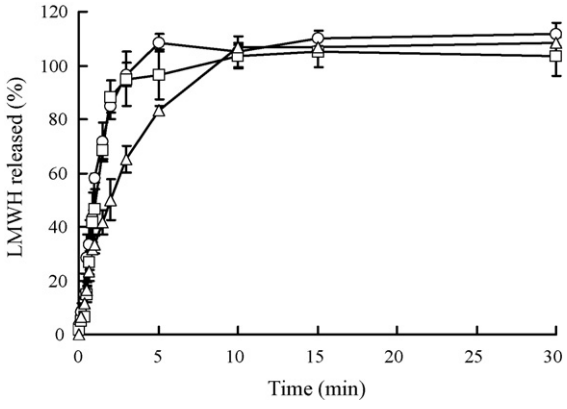

Fig. 4. *In vitro* release profiles of LMWH from SDNs. (◯) Dextran; (□) chondroitin; (△) dextrin. Each point shows the mean ± S.E. of three experiments.

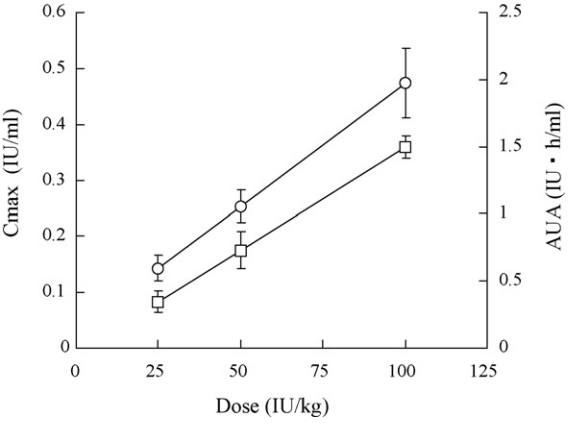

Fig. 6. Relations between C_{max} (◯) or AUA (□) and LMWH dose. Each point shows the mean ± S.E. of three experiments.

Table 2
Stability of LMWH in SDNs

Temperature (°C)	Remaining of LMWH (%)	
	1 month	3 months
40	97.49 ± 1.05	98.65 ± 2.42
4	98.89 ± 2.07	97.68 ± 2.36
−80	97.49 ± 2.09	99.94 ± 1.64

Each value represents the mean ± S.E. of three experiments.

fore, dose-dependent plasma anti-Xa activity was obtained by LMWH SDNs.

Finally, the stability of LMWH in dextran SDNs was studied for 3 months and the results are shown in Table 2. Three conditions, −80, 4 and 40 °C, were used. In this experiment, the humidity was zero by measuring in the incubator (40 °C) and the refrigerator (4 °C). With respect to deep freezer used for −80 °C experiment, we could not measure humidity by our tester. However, there were not significant differences on the LMWH contents between the three conditions. In addition, there were also not significant differences on LMWH contents between 1 and 3 months. Therefore, we may state that LMWH is stable in SDN preparation.

4. Discussion

Based on the development of micro-fabrication technology, the use of micron-scale needles showed a dramatical increase of the skin permeability of drugs. Namely, solid microneedles made of stainless steel have been shown to increase the transdermal permeability of drugs (Kim et al., 2004; Chandrasekaran et al., 2003; Griss et al., 2002). Therefore, microneedles of which size is microns dimensions has been given much attention for the transdermal delivery of drugs having poor membrane permeability like peptide/protein drugs and polysaccharide.

In this study, LMWH was used as a model polysaccharide and percutaneous administration of LMWH using SDNs has been challenged. From the results on the plasma anti-Xa activity *vs*. time profiles, C_{max} appeared at about 1 h after percutaneous administration of LMWH SDNs. From the standpoint of pharmacokinetic analysis, LMWH was absorbed with an acceptable rate from SDN. The plasma anti-Xa activity *vs*. time profiles were compared between the used three polymers as the base. Dextrin SDNs showed slow absorption rate and *in vitro* release rate. There were significant differences between the used three polymers *in vitro* study, though LMWH was completely released from the all SDNs within 10 min. Therefore, we mat state that the base to make SDN is not the critical factor for the PA of LMWH from SDNs.

In mechatronics field, silicon or metal was used for fabrication (Kim et al., 2004; Chandrasekaran et al., 2003; Griss et al., 2002; Zahn et al., 2000; Teo et al., 2005). However, it is difficult to use these materials as pharmaceutical base or additive because of the safety problem. Therefore, three biodegradable and thread-forming polymers, dextrin, chondroitin and dextran, were used in our SDNs. These polymers are endogenous compounds and toxicity will not be a problem to develop SDN as a new percutaneous DDS. In general, as a biodegradable polymer, polylactic acid, poly glycocholic acid and their copolymers have been used as DDS wall materials (Ambrose and Clanto, 2004; Park et al., 2006). However, the degradation rate of these polymers is slow. On the other hand, several TTSs are clinically used, though their clinical use is the treatment of chronic diseases like hypertension, asthma and postmesonal disease, etc. In the case of LMWH, short release time is required to simulate the plasma anti-Xa activity *vs*. time profile that is obtained after subcutaneous injection of LMWH solution that resulted in the increase of anti-Xa activity. Therefore, water-soluble polymers that dissolved rapidly within the body were used in this study.

5. Conclusion

A new self-dissolving needles (SDNs) containing LMWH have been prepared for the percutaneous administration of LMWH. After administration of LMWH SDNs, plasma ant-Xa activity *vs*. time profiles were studied with three thread-forming polymers, i.e., dextrin, chondroitin and dextran. The C_{max} were 0.40 ± 0.03, 0.46 ± 0.03 and 0.47 ± 0.06 IU/ml. AUAs were 1.56 ± 0.23, 1.24 ± 0.10 and 1.49 ± 0.08 IU h/ml. T_{max} were 1.67, 0.83 and 1.00 h. The physiological availabilities were 102.3%, 81.5% and 97.7%, respectively. Plasma anti-Xa activity *vs*. time profiles showed a good dose-dependency. These results support the usefulness of SDNs for the percutaneous administration of polysaccharide drugs like LMWH.

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