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Dermatopharmacokinetics of salicylate following topical injection in rats: Effect of osmotic pressure and injection volume on salicylate disposition

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Abstract

Using advanced topical formulations containing potential chemical enhancer(s) or physical penetration-enhancing tools capable of delivering entrapped drug(s) directly into skin tissues with little influence of the stratum corneum barrier, local and systemic drug disposition may be markedly similar to direct injection into the skin and muscle. The objective of this study is to investigate the dermatopharmacokinetics and systemic drug disposition after topical application and topical injection. Salicylate (SA) disposition in the skin and muscle as administration sites, and in the systemic circulation were evaluated following intracutaneous (i.c.) injection of an isotonic solution of SA-Na (dose; 3.08 μmol). Subcutaneous (s.c.) and intramuscular (i.m.) injection were also evaluated for comparison. Dermatopharmacokinetics and systemic disposition of SA after i.c. and s.c. injections were analyzed using a 4-compartment model consisting of skin, muscle, and central and peripheral compartments, whereas SA disposition after i.m. injection was analyzed using a 3-compartment model consisting of muscle, and central and peripheral compartments. Moreover, the absorption rate constant of SA after i.c. injection (0.073 min^{-1}) was slightly lower than that after s.c. injection (0.083 min^{-1}), and much lower than that after i.m. injection (0.327 min^{-1}). In addition, higher osmolarity and a larger volume of SA-Na injectant increased the retention of SA in the skin and decreased the absorption rate to the systemic circulation after i.c. injection. The effect of injection volume on SA disposition after i.c. injection was not so marked compared with that of osmotic pressure. These results are useful to design an injection-type topical delivery system.

Keywords: Dermatopharmacokinetics; Salicylate; Injection; Osmotic pressure; Injection volume

1. Introduction

Technologies for chemical enhancers and the application of dermatopharmacokinetics (DPK) are very important to establish a good topical therapeutic system to effectively deliver drugs to the skin or muscle as a target site. DPK after passage of the stratum corneum has been investigated by various methods using a multi-compartment model (Singh and Roberts, 1994; Nakayama et al., 1999), microdialysis (Seki et al., 2004), an in-situ absorption experiment using agar gel (Yanagimoto et al., 1999), and so on.

These penetration-enhancing methods are classified into two categories, chemical and physical. The former are methods using chemical enhancers (Ogiso et al., 1995), prodrugs (Sloan et al., 1983) and the formation of drug complex (Valenta et al., 2000). The latter are exemplified by phonophoresis (Mitragotri et al., 1995), electroporation (Prausnitz et al., 1993; Tokudome and Sugibayashi, 2003) and iontophoresis (Tyle, 1986), and several combinations of these methods (Fang et al., 2004; Tokumoto et al., 2005). Other physical means using non-needle injection (Inoue et al., 1996; Baxter and Mitragotri, 2005) and a microneedle system (Prausnitz, 2004; Wu et al., 2006) were developed as a way to directly load drugs into the skin. The advantages of these methods are possible self-injection and less pain during administration. Non-needle injection is the principle for administering drug solution into skin tissues by positive pressure. Insulin and growth hormone delivery systems with a non-needle syringe have already been used in the clinical field. Array devices consisting of solid and hollow microneedles several hundred microns in length coated with drugs have also been developed to deliver macromolecular drugs such as peptide and DNA (Birchall et al., 2005) into the skin. The delivery routes

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for these devices (microneedles and needle-free injection) are almost the same as for conventional topical injection. These delivery systems have a drug depot in the skin and muscle, as in topical injection. Thus, the DPK after the application of such new transdermal delivery systems may be different from that of conventional topical application.

Although systemic pharmacokinetics after s.c. or i.m. injection of drugs has been studied for a long time (Kakemi et al., 1969, 1971), the objective of this study was to identify the absorption rate of drugs from the injection sites into the systemic circulation and systemic bioavailability. Thus, we evaluated the DPK and systemic disposition after topical injection as a model of new transdermal delivery systems. In our previous report (Yoshida et al., 2002), i.c. injection of SA-Na solution, as well as s.c. and i.m. injections, were performed in the rat abdomen to evaluate salicylate (SA) dispositions. A high dose (high osmotic pressure) was injected into the skin or muscle to observe the differences and characteristics of DPK after topical injections. Pharmacokinetic analysis was performed using a 4-compartment model consisting of skin, muscle, and central and peripheral compartments. As a result, SA was absorbed through the muscle vessels as well as through the systemic circulation in the skin after i.c. and s.c. injections. Thus, SA retention in the muscle following i.c. and s.c. injections was more prolonged than that following i.m. injection.

In this report, an isotonic solution of SA-Na was loaded into skin or muscle by i.c. and s.c., or i.m. injection to confirm whether the retention time in the muscle after i.c. and s.c. injections was longer than that after i.m. injection. SA disposition was estimated in the skin and muscle below without changing the osmotic pressure of the injectant at the injection site during these experiments. Additionally, the effects of injection volume and osmotic pressure were also evaluated in drug disposition following i.c. injection.

2. Materials and methods

2.1. Materials

SA-Na and D-glucose were supplied by Wako Pure Chemical Industries (Osaka, Japan). Other chemicals and solvents were of reagent grade and were used without further purification.

2.2. Animals

Male Wistar rats, weighing of 300 ± 20 g, were purchased from Saitama Experimental Animals (Sugito, Saitama, Japan). Rats were anesthetized with urethane (1.0 g/kg, i.p.) and the hair on their abdomen was shaved. Their body temperature was maintained at $36.5 \pm 0.5\,^{\circ}C$ throughout the experiments. All animal experiments were performed in accordance with the guidelines of the Life Science Research Center, Josai University.

2.3. Drug preparations

Table 1 shows the injected tissue and properties of several SA-Na solutions. Isotonic SA-Na solution (308 mOsm) was prepared for administration (dose; 3.08 μmol) into the skin (by i.c., s.c. injection) or the muscle (Nos. 1–3). SA-Na was added to the preparations to adjust the osmotic pressure of the injectants. Hypertonic solutions (2893 mOsm) were also prepared by adding D-glucose to evaluate the effect of osmotic pressure (No. 4) and injection volume (No. 5) on SA disposition following i.c. injection.

2.4. i.c., s.c. and i.m. injection

Several solutions of SA-Na were loaded into the abdominal site in rats by i.c., s.c. and i.m. injection with a 27 G needle. In i.c. and s.c. injections, the drug solution was loaded at approximately the same position in skin without fixing the depth from the skin surface. For i.m. injection, the tip of the needle was inserted to a depth of 2 mm below the skin surface. After dosing, blood samples were periodically collected from the jugular vein and centrifuged (15,000 rpm, 5 min, 4 °C) to obtain plasma. Skin and muscle tissues (3 cm diameter) containing subcutaneous tissue were excised at the end (various times) of the experiment. The samples were frozen and stored at −20 °C until analysis. In this study, the amount of SA in skin and muscle was that within a circle 3 cm in diameter.

2.5. Salicylate assay

Plasma samples were mixed with a two-fold volume of acetonitrile containing propyl *p*-hydroxybenzoate as an internal standard, and centrifuged to obtain the supernatant. Excised skin and muscle were homogenized with 4.0 mL each of physiological saline and acetonitrile containing the internal standard, and centrifuged. The resulting supernatant samples were injected into an HPLC column. The HPLC system consisted of a pump (LC-10AS, Shimadzu, Kyoto, Japan), a UV detector (SPD-10A, Shimadzu), an integrator (C-R5A, Shimadzu), a column oven (CTO-6A, Shimadzu), a system controller (SCL-10A, Shimadzu), an auto injector (Sil-10Axl, Shimadzu), and

Table 1
Route of administration and preparation of injectant

Injectant No.	Route of administration	Dose (μmol)	Volume (μL)	D-Glucose (μmol)	Osmotic pressure (mOsm)
1	i.c.	3.08	20	–	308
2	s.c.	3.08	20	–	308
3	i.m.	3.08	20	–	308
4	i.c.	3.08	20	51.7	2893
5	i.c.	3.08	5	8.31	2893

a reverse-phase column (LiChrospher 100, RP-18e (5 μm) 250-4, Darmstadt, Merck, Germany). The mobile phase was 0.1% phosphoric acid–methanol (45:55, v/v) and the flow rate was 0.8 mL/min. The UV detector was operated at 225 nm and column temperature was maintained at 40 °C. The coefficient of variation (CV) for each standard curve ranged from 0.8 to 3.5% and the squared correlation coefficient was over 0.999.

3. Theoretical

Model pharmacokinetic analysis using a multi-compartment model has been performed by Singh and Roberts (1994) and Higaki et al. (2002) after topical application. Both methods are suitable to understand the pharmacokinetics of topically applied drugs, especially to quantitatively assess the migration rate from one organ to another. Increasing the number of compartments in the pharmacokinetic model is sometimes problematic as there are more data points. As our models were a little complicated and composed of many parameters, the drug disposition in each tissue following topical injections was analyzed by fixing the elimination parameters obtained by intravenous injection. The difference in drug absorption after various injections as well as the influence of osmotic pressure and volume of drug solution were evaluated using the obtained pharmacokinetic parameters by this model analysis. Fig. 1 shows several compartment models composed of skin, muscle, and central and peripheral compartments. In the i.c. and s.c. injection, and i.m. injection, the administered compartment is skin (Fig. 1a) and muscle (Fig. 1b), respectively. In our previous report (Yoshida et al., 2002), it was confirmed that no direct migration of SA from the muscle to skin was observed and most of the drug in the skin had migrated from the systemic circulation following i.m. injection. Since the k_{ms} value converged to almost zero in a preliminary analysis, the value was fixed at zero in this paper.

The migration rates between compartments are expressed as follows:

In the i.c. and s.c. injection

$$S: \quad \frac{dS}{dt} = -(k_{sm} + k_{sc})S + k_{ms}M + k_{cs}C \tag{1}$$

$$M: \quad \frac{dM}{dt} = k_{sm}S - (k_{ms} + k_{mc})M + k_{cm}C \tag{2}$$

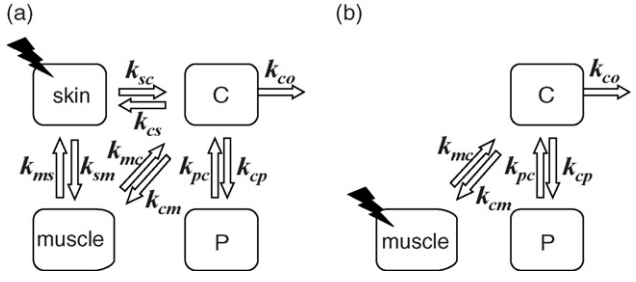

Fig. 1. Pharmacokinetic models for SA following i.c. and s.c. injections (a) and i.m. injection (b). C, central compartment; P, peripheral compartment.

$$C: \quad \frac{dC}{dt} = k_{sc}S + k_{mc}M - (k_{cs} + k_{cm} + k_{cp} + k_{co})C + k_{pc}P \tag{3}$$

$$P: \quad \frac{dP}{dt} = k_{cp}C - k_{pc}P \tag{4}$$

In the i.m. injection

$$M: \quad \frac{dM}{dt} = -k_{mc}M + k_{cm}C \tag{5}$$

$$C: \quad \frac{dC}{dt} = k_{mc}M - (k_{cm} + k_{cp} + k_{co})C + k_{pc}P \tag{6}$$

$$P: \quad \frac{dP}{dt} = k_{cp}C - k_{pc}P \tag{7}$$

where S, M, C and P are the skin, muscle, central and peripheral compartments, respectively. The obtained rate constants are summarized in Fig. 1.

Eqs. (1)–(7) can be changed to the following difference equations.

In the i.c. and s.c. injection

$$S: \quad S_{i+1} = \{-(k_{sm} + k_{sc})S_i + k_{ms}M_i + k_{cs}C_i\}\Delta t + S_i \tag{1'}$$

$$M: \quad M_{i+1} = \{k_{sm}S_i - (k_{ms} + k_{mc})M_i + k_{cm}C_i\}\Delta t + M_i \tag{2'}$$

$$C: \quad C_{i+1} = \{k_{sc}S_i + k_{mc}M_i - (k_{cs} + k_{cm} + k_{cp} + k_{co})C_i + k_{pc}P_i\}\Delta t + C_i \tag{3'}$$

$$P: \quad P_{i+1} = (k_{cp}C_i - k_{pc}P_i)\Delta t + P_i \tag{4'}$$

In the i.m. injection

$$M: \quad M_{i+1} = \{-k_{mc}M_i + k_{cm}C_i\}\Delta t + M_i \tag{5'}$$

$$C: \quad C_{i+1} = \{k_{mc}M_i - (k_{cm} + k_{cp} + k_{co})C_i + k_{pc}P_i\}\Delta t + C_i \tag{6'}$$

$$P: \quad P_{i+1} = (k_{cp}C_i - k_{pc}P_i)\Delta t + P_i \tag{7'}$$

where S_i, M_i, C_i and P_i are the drug amounts in each tissue at ith time. Δt is $t_{i+1} - t_i$. Initial conditions were as follows:

In the i.c. and s.c. injection

$$S_0 = \text{Dose}, \qquad M_0 = C_0 = P_0 = 0 \quad \text{at } t = 0$$

In the i.m. injection

$$M_0 = \text{Dose}, \qquad C_0 = P_0 = 0 \quad \text{at } t = 0$$

SA amounts in the tissues and plasma were fitted to these difference equations with the Solver function of Microsoft Excel by the nonlinear least-squares method (algorithm; quasi-Newton method).

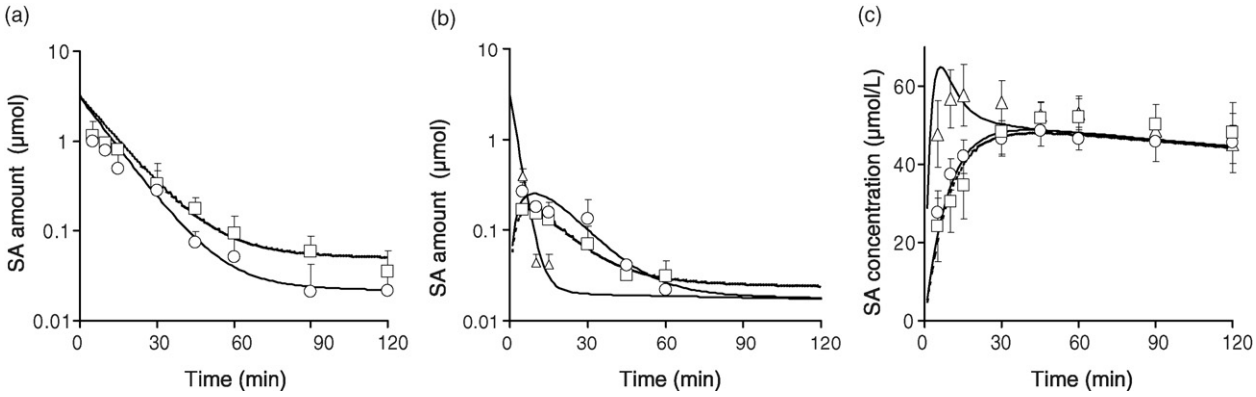

Fig. 2. Time courses of SA amount in the skin (a) and muscle (b), and plasma concentration (c) following topical injections of SA-Na (3.08 μmol/20 μL). Symbols: (□), i.c. injection (Table 1, No. 1); (◯), s.c. injection (Table 1, No. 2); (△), i.m. injection (Table 1, No. 3). Each line was obtained by curve fitting. Each data point represents the mean ± S.E. of 3–6 experiments.

4. Results and discussion

4.1. The DPK and systemic disposition following topical injections

Fig. 2a and b shows the time courses of SA amounts in skin and muscle, respectively, and Fig. 2c shows the time course of SA concentration in plasma. SA in the muscle was under the determination limit (less than 0.02 μmol) at 90 min after i.c. and s.c. injection. Generally, these data are markedly different from those during the topical application of drugs: the drug in the skin gradually increases and plasma concentration reaches a steady-state level after a relatively long period of time (*i.e.*, a few hours).

Following i.m. injection, rapid migration of SA from the muscle to the systemic circulation was observed. Plasma concentration of SA was decreased and the amount in the muscle was under the determination limit at 30 min after i.m. injection, suggesting that the absorption process was completed within 30 min. On the other hand, prolonged retention of SA concentration in the skin was observed after i.c. and s.c. injections, probably due to the slow transfer of SA from the injection site to the systemic circulation. The obtained SA data were fitted to the compartment model (Fig. 1) by the nonlinear least-squares method (see Section 3). In i.c. and s.c. injections, better agreement of actual and theoretical values was obtained by assuming that the rate constant k_{ms} from the muscle to skin was zero. Unfortunately, the initial plasma concentration of SA after i.m. injection was not well fitted to the theoretical curve. This was partially due to simultaneous curve-fitting of the SA amount in the systemic circulation and muscle. The SS (sum of squares) value of the least-squares method was smallest among several analyses. No clear explanation was obtained for this.

The calculated pharmacokinetic parameters following these topical injections are summarized in Table 2. SA absorption to the systemic circulation after i.c. and s.c. injections was obtained through the skin vessels (k_{sc}) and muscle vessels (k_{mc}); however, regarding the elimination rate of SA from the muscle, the migration rate from skin to muscle (k_{sm}) was much slower than the absorption rate from the muscle to systemic circulation. In other words, k_{sm} is thought to be a constant for the rate-controlling step in absorption from the muscle. Therefore, we assumed k_s (the sum of k_{sc} and k_{sm}) and k_{mc} as the absorption rate constant to the systemic circulation in the case of i.c. and s.c. injection, and i.m. injection, respectively. The absorption rate constant of SA after i.c. injection (0.073 min^{-1}) was slightly lower than that after s.c. injection (0.083 min^{-1}), and much lower than that after i.m. injection (0.327 min^{-1}), as the blood vessels are richer in muscle and subcutaneous tissues than in cutaneous tissues. The distance between the injection site and blood vessels, and local blood flow are very important when discussing the absorption rate and drug processing with microneedle delivery systems. Furthermore, the percent direct migration ratio from skin to muscle was expressed by $k_{sm}/k_s \times 100$. The ratios after i.c. and s.c. injection were both 26%. These values were higher than the direct migration ratio from skin to muscle after topical application (Yanagimoto et al., 1999).

In our previous report (Yoshida et al., 2002), a high dose (62.46 μmol/20 μL) of SA-Na as a hypertonic solution was used to clarify pharmacokinetic profiles at different injection sites. In this report, on the other hand, we used an isotonic solution of SA-Na (3.08 μmol/20 μL) and performed a similar experiment

Table 2
Pharmacokinetic parameters following topical injections

Injectant No.	Route of administration	k_{sc} (min^{-1})	k_{sm} (min^{-1})	k_s (min^{-1})	k_{cs} (min^{-1})	k_{mc} (min^{-1})	k_{cm} (min^{-1})
1	i.c.	0.054	0.019	0.073	0.002	0.220	0.003
2	s.c.	0.061	0.022	0.083	0.001	0.129	0.001
3	i.m.	–	–	–	–	0.327	0.004
4	i.c.	0.005	0.026	0.031	0.003	0.282	0.008
5	i.c.	0.005	0.036	0.041	0.005	0.396	0.012

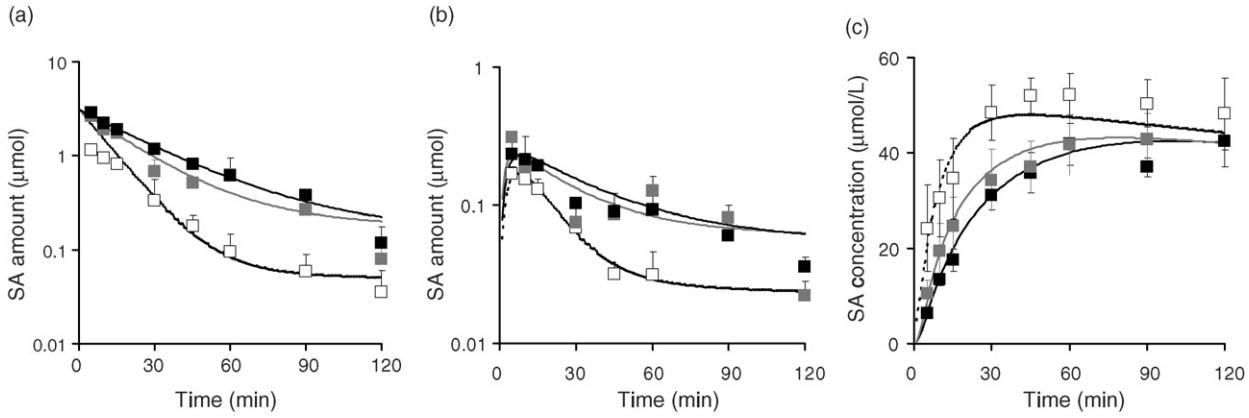

Fig. 3. Effect of osmotic pressure and injection volume on SA amount in the skin (a) and muscle (b), and plasma concentration (c) following i.c. injection of SA-Na. (□) 308 mOsm, 20 μL (Table 1, No. 1); (■) 2893 mOsm, 20 μL (Table 1, No. 4); (■) 2893 mOsm, 5 μL (Table 1, No. 5). Each line was obtained by curve fitting. Each data point represents the mean ± S.E. of 3–6 experiments.

so as not to change osmotic pressure in the skin during the experiment. Therefore, we improved our previous model and re-evaluated the DPK and systemic disposition after injection of SA-Na. Rapid absorption to the systemic circulation and short retention of the drug at the injection site following topical injections of isotonic solution compared with following hypertonic solution were confirmed.

4.2. Effect of osmotic pressure and injection volume on SA disposition following i.c. injection

Each solution of i.c. injection is shown in Table 1 (Nos. 1, 4 and 5). D-Glucose was added to increase osmotic pressure in injectants, Nos. 4 and 5.

4.2.1. Effect of osmotic pressure

To evaluate the effect of osmotic pressure, isotonic (No. 1; 308 mOsm) and hypertonic (No. 4; 2893 mOsm) solutions of SA-Na (3.08 μmol) were loaded into the skin by i.c. injection. The time courses of SA amounts in the skin and muscle, and SA concentrations in plasma following i.c. injection are shown in Fig. 3a–c,, respectively. SA retention in the skin after i.c. injection of hypertonic solution was much more prolonged than that of isotonic solution. According to pharmacokinetics analysis, k_{sm} after i.c. injection of hypertonic solution of SA was not different from that after injection of isotonic solution, whereas k_{sc} for hypertonic solution became extremely low compared with that for isotonic solution. Drug migration from skin to muscle must be due to simple diffusion, but water movement is also related to SA migration from the skin to the systemic circulation. When hypertonic solution was loaded into the skin by i.c. and s.c. injection, an injectant depot was formed and edema developed. These processes probably come from water influx from the surrounding tissues to the depot. This water influx may decrease SA migration from the depot to skin vessels, so that SA retention in the skin may be prolonged. Plasma concentration profiles of SA indicate more rapid drug absorption to the systemic circulation after injection of isotonic solution compared with hypertonic solution. However, the SA solution spread throughout epidermis and dermis did not affect SA migration to muscle, because the solution was located in contact with subcutaneous tissue and muscle. Thus, k_{sm} became larger as an alternate route to decrease drug absorption to blood vessels.

4.2.2. Effect of injection volume

To evaluate the effect of injection volume, a higher (No. 4; 20 μL) or a lower (No. 5; 5 μL) volume of SA-Na (3.08 μmol) was loaded into the skin by i.c. injection. When an isotonic solution of SA-Na was administered with an injection volume of 5 μL, the SA amount migrating to the muscle or systemic circulation was under the limit of determination; therefore, the effect of injection volume was compared using a hypertonic solution. The time courses of the SA amount in the skin and muscle, and plasma concentration following i.c. injection are shown in Fig. 3a–c, respectively. SA retention in the skin after i.c. injection of a higher volume was slightly more prolonged than after injection of a lower volume. The rate constant k_s obtained by the present model analysis indicates that elimination from the skin or absorption to the systemic circulation after i.c. injection of higher volume was slower than that of lower volume (Table 2). However, the effect of the injection volume was not so large compared with that of osmotic pressure, since SA migration from skin to systemic circulation was more decreased after i.c. injection of hypertonic solution. The direct migration ratios from the skin to muscle after i.c. injection of No. 4 and No. 5 formulations were about 84% and 88%, respectively. The effect of osmotic pressure was more marked for drug migration to muscle from skin than that after No. 1 formulation.

5. Conclusions

Since rapid migration of SA to the muscle located beneath the injection site was observed following i.c. and s.c. injections of SA-Na, distribution equilibrium was established in the drug amount in the skin and muscle immediately after drug administration. Osmotic pressure and injection volume can be utilized to control the drug migration rate from the injection site, especially with i.c. injection. DPK was very different from that after

conventional transdermal application. The osmolality and delivery rate of the solvent are very important factors to control the drug delivery rate from enhanced transdermal delivery systems, especially from non-needle and microneedle delivery systems. These findings can be applied to develop a new type of enhanced transdermal delivery system.

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