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# Effect of vasoactive agents on the dermatopharmacokinetics and systemic disposition of model compounds, salicylate and FITC-dextran 4 kDa, following intracutaneous injection of the compounds

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**Abstract**

The effects of two vasoactive agents, phenylephrine and tolazoline, were determined on the dermatopharmacokinetics and systemic disposition of model compounds, salicylate (SA) and FITC-dextran 4 kDa (FD-4), following their intracutaneous (i.c.) injection. The determined blood flow in skin was lowered and increased by i.c. injection of phenylephrine and tolazoline, respectively. Dermatopharmacokinetics and the systemic disposition of SA and FD-4 with and without vasoactive agents were analyzed using a compartment model. As a result, the rate constant, $k_{sc}$, from skin to systemic circulation of SA after i.c. injection with phenylephrine was almost zero, and the rate constant, $k_{sm}$, from skin to muscle increased about 2.4-fold compared with the control group (without vasoactive agents). In contrast, the rate constants, $k_{sc}$ and $k_{sm}$, after i.c. injection of SA with tolazoline were increased about 1.9- and 2.5-fold, respectively, compared with the control. In FD-4 disposition, $k_{sc}$ and $k_{sm}$ decreased to about 0.3-fold and increased to about 4.0-fold compared with the control after i.c. injection with phenylephrine. The $k_{sc}$ and $k_{sm}$ of FD-4 increased with tolazoline about 2.2- and 4.3-fold compared with the control, respectively. These data suggest that these vasoactive agents can be used to modify the dermatopharmacokinetics of topically or intracutaneously applied drugs.


*Keywords:* Vasoactive agent; Intracutaneous injection; Salicylate; FITC-dextran; Dermatopharmacokinetics

## 1. Introduction

Recently, various transdermal drug delivery devices have been developed to bypass the permeation barrier of the stratum corneum and to deliver drugs directly into skin tissues. For instance, non-needle syringes are utilized to deliver insulin and growth hormone to patients (Verrips et al., 1998; Bremseth and Pass, 2001). A spraying device containing estradiol named Estradiol MDTS® has been developed by Acrux (West Melbourne, VIC, Australia) where EvaMist™ produces high pressure to administer the drug directly into the stratum corneum and viable epidermis (Finnin and Hadgraft, 2006). Microneedle arrays are also utilized to deliver drugs transdermally, with many microscale needles in two dimensions. Solid and hollow needles have already been designed (Teo et al., 2005), made of metal (Martanto et al., 2004), melted sugar (Miyano et al., 2005) or biodegradable polymer (Park et al., 2006). They can be applied like dermal patches, but they are not conventional dermal patches but intracutaneous (i.c.) injectors. As these technologies are different from conventional transdermal formulations and completely avoid the stratum corneum barrier, it is believed that these devices will be the next generation of topical formulations.

We have already analyzed dermatopharmacokinetics after i.c. infusion of drug solution to each depth of the skin membrane to evaluate the utility of these types of delivery systems (Yoshida et al., 2002). As a result, i.c. injection is very useful to deliver drugs to the systemic circulation and underlying muscle from the application site of skin, although it is not a common administration method for therapeutic drugs except for tuberculin reaction and diagnosis of anaphylaxis reaction. The dermatopharmacokinet-

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ics after injection was greatly affected by cutaneous blood flow at the injection site as well as the osmotic pressure of the applied solution. Thus, the physicochemical properties of applied drugs (*i.e.*, molecular weight and protein binding), injection medium (*i.e.*, volume and osmolarity) and body environment (*i.e.*, cutaneous temperature and blood flow) must have a great influence on the dermatopharmacokinetics after direct injection into cutaneous tissues.

Skin permeation of topically applied drugs can be increased by several chemical enhancers (Obata et al., 2000) and percutaneous absorption of the drugs (uptake into the systemic circulation) can be regulated by vasoactive agents (Higaki et al., 2005) as well as chemical enhancers. It is clear that the stratum corneum must be the biggest barrier against the skin permeation of drugs. When the drugs are administrated by evading the stratum corneum barrier; *i.e.*, by i.c. injection, the effect of vasoactive agents may be much higher on the drug absorption into the systemic circulation. In the present study, therefore, we paid attention to the blood flow in skin. We have reported (Sugibayashi et al., 1999) that drug absorption to the systemic circulation after topical application was inhibited by a vasoconstrictor agent, epinephrine. It was also reported (Singh and Roberts, 1994; Bernards and Kopacz, 1999) that epinephrine or phenylephrine was applied at the same time to prolong the retention time of lidocaine in the tissues. Since the effect of vasoactive agents must be greater after i.c. injection than after topical application, the combination with vasoactive agents provides more suitable properties for drug targeting and delivery after i.c. injection. Phenylephrine and tolazoline were used as a model α-blocker and α-agonist, respectively, to change cutaneous blood flow after i.c. injection in the present study. Sodium salicylate (SA; MW 160) and fluorescein isothiocyanyte-labeled dextran 4 kDa (FD-4; MW 3,820) were selected as model drugs, and dermatopharmacokinetics and systemic disposition after the i.c. injection of SA and FD-4 were determined with and without combination with vasoactive agents.

## 2. Materials and methods

### 2.1. Materials

Sodium salicylate and tolazoline HCl were supplied by Wako Pure Chemical Industries (Osaka, Japan). FD-4 and phenylephrine HCl were supplied by Sigma–Aldrich (St. Louis, Missouri, U.S.A.). 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). The 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 ± 0.5 °C throughout the experiments. All animal experiments were performed in accordance with the guidelines of the Life Science Research Center, Josai University (Sakado, Saitama, Japan).

### 2.3. Measurement of cutaneous blood flow

Cutaneous blood flow was noninvasively determined by Peri Scan PIM II laser Doppler perfusion imager (Perimed AB, Stockholm, Sweden). A circle of 1.0 cm diameter, including the center of the drug depot formed in the skin, was irradiated with laser light to measure blood flow using a laser doppler perfusion imager at predetermined times after i.c. injection of phenylephrine (50 nmol/20 μL phosphate-buffered saline, PBS) or tolazoline (2 nmol/20 μL PBS).

### 2.4. Intravenous injection

FD-4 (1.31 μmol/kg) was intravenously injected into the jugular vein of rats, and blood samples were collected from the contralateral jugular vein at predetermined times. The resulting blood samples were centrifuged (15,000 rpm, 5 min, 4 °C) to obtain plasma.

### 2.5. Intracutaneous injection

Several drug solutions (SA-Na; 3.08 μmol/20 μL purified water and FD-4; 78.5 nmol/20 μL PBS) were intracutaneously loaded using a 27G needle into the abdominal site in rats. Both drug solutions were injected with and without phenylephrine (50 nmol) or tolazoline (2.0 nmol). The drug solution could be loaded to approximately the same position in the skin without fixing the depth from the skin surface. After dosing, blood samples were periodically collected from the jugular vein and centrifuged to obtain plasma. Skin and muscle tissues (3.0 cm in diameter) containing subcutaneous tissue were excised at the end of experiments (experimental period differed). The biological samples were frozen and stored at −20 °C until analysis. In this study, the drug amount in skin and muscle was represented as that within a circle of 3.0 cm diameter.

### 2.6. SA assay

Plasma samples were mixed with two-times 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 supernatants were injected into an HPLC. The HPLC system consisted of a pump (LC-10AS, Shimadzu, Kyoto, Japan), UV detector (SPD-10A, Shimadzu), integrator (C-R5A, Shimadzu), column oven (CTO-10A, Shimadzu), system controller (SCL-10A, Shimadzu), autoinjector (Sil-10Axl, Shimadzu), and 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 from 2.5 to 50 μM ranged from 0.8 to 3.5% and the correlation coefficient was over 0.999.

### 2.7. FD-4 assay

Plasma samples were mixed with four-times volume of PBS, and centrifuged (15,000 rpm, 5 min, 4 °C) to obtain the supernatant. Excised skin and muscle were homogenized with 8.0 mL each of physiological saline, and centrifuged to obtain the supernatant. Each sample was measured at an excitation wavelength of 495 nm and an emission wavelength of 515 nm using a spectrophotofluorometer (RF-5300 PC, Shimadzu). The coefficient of variation (CV) for each standard curve from 12.5 to 250 nM ranged from 1.0 to 3.9% and the correlation coefficient was over 0.999.

## 3. Theoretical

In our previous report, the effect of the osmotic pressure of solution and injection volume on the dermatopharmacokinetics and systemic disposition of SA after i.c. injection was estimated using a compartment model (Fig. 1) consisting of skin, muscle, and central and peripheral compartments (Yoshida et al., 2007). These methods are suitable to understand the pharmacokinetics of topically applied drugs, especially to quantitatively assess the migration rate from one organ to another. Changes of dermatopharmacokinetics and systemic disposition of SA and FD-4 after i.c. injection were evaluated using this compartment model analysis.

Migration rates between compartments are expressed as follows:

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

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

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

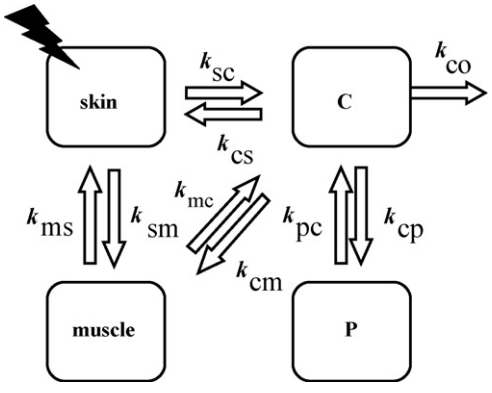


Fig. 1. Pharmacokinetic models for the dermato- and systemic disposition of drugs following i.c. injection. Abbreviations: C, central compartment; P, peripheral compartment. $k_{sm}$, $k_{ms}$, $k_{sc}$, $k_{cs}$ $k_{mc}$, $k_{cm}$, $k_{cp}$, $k_{pc}$ and $k_{co}$ are first order rate constants from skin to muscle, from muscle to skin, from skin to central, from central to skin, from muscle to central, from central to muscle, from central to peripheral, from peripheral to central and from central to outside, respectively.

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

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

Eqs. (1)–(4) can be changed to the following difference equations:

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

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

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

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

where $S_i$, $M_i$, $C_i$ and $P_i$ are drug amounts in each tissue at $i$-th time. $\Delta t$ is $t_{i+1} - t_i$. Initial conditions were as follows: $S_0$ = dose, $M_0 = C_0 = P_0 = 0$ at $t = 0$.

Average drug amounts in tissues and plasma were simultaneously fitted to these difference equations with the solver function of Microsoft® Excel by the nonlinear least-squares method (Algorithm; quasi–Newton method). The drug disposition in each tissue following topical injections was analyzed by utilizing the elimination parameters obtained by intravenous injection. Since the $k_{ms}$ value converged to almost zero in preliminary analysis, the value was fixed at zero in this paper.

## 4. Results

### 4.1. Cutaneous blood flow following i.c. injection of vasoactive agents

Fig. 2 shows the time courses of cutaneous blood flow after i.c. injection of vasoactive agents/PBS. The vertical axis shows

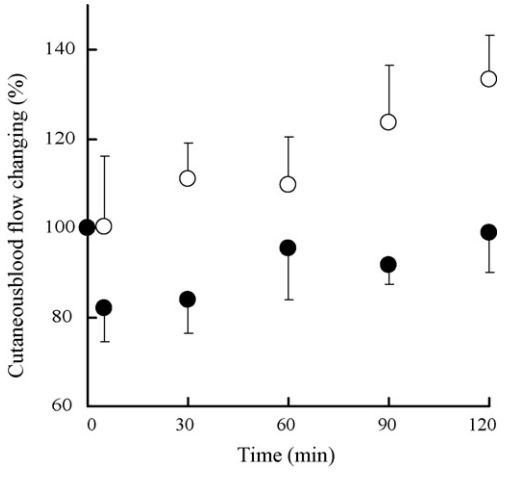


Fig. 2. Time courses of cutaneous blood flow following i.c. injection of vasoactive agents. Symbols: (●), phenylephrine (2 nmol/20 μL); (○), tolazoline (50 nmol/20 μL). Each data point represents the mean ± S.E. of three to four experiments.

Table 1
Pharmacokinetic parameters following intravenous injection

| Drugs | $k_{cp}$ ($min^{-1}$) | $k_{pc}$ ($min^{-1}$) | $k_{co}$ ($min^{-1}$) | $V_1$ (mL/kg) | $V_2$ (mL/kg) |
|---|---|---|---|---|---|
| SA[a] | $(6.3 \pm 0.3) \times 10^{-2}$ | $(9.3 \pm 0.3) \times 10^{-2}$ | $(2.2 \pm 0.3) \times 10^{-3}$ | 124.3 ± 7.1 | 73.3 ± 6.2 |
| FD-4 | $(4.5 \pm 0.9) \times 10^{-2}$ | $(4.1 \pm 0.7) \times 10^{-2}$ | $(3.1 \pm 0.4) \times 10^{-2}$ | 134.2 ± 7.7 | 145.3 ± 7.8 |

Each data value represents the mean ± S.E. of three experiments.
[a] SA parameters were referred from our previous report (Yoshida et al., 2002).

% of the cutaneous blood flow at time *t* against that before administration. No change in cutaneous blood flow was observed, being similar to non-treatment of i.c. saline injection. In contrast, blood flow decreased within 5 min after i.c. injection of phenylephrine, and returned to the value before administration within 2 h. On the other hand, blood flow slowly increased for 2 h after i.c. injection of tolazoline, and did not return to the control blood flow before administration in this experiment period.

### 4.2. Elimination kinetics following i.v. injection

Intravenous (i.v.) injection study was conducted to obtain the elimination and distribution pharmacokinetic parameters of drugs in rats. Table 1 summarizes the pharmacokinetics parameters of SA and FD-4 after i.v. injection SA (39.02 μmol/kg) and FD-4 (1.31 μmol/kg). Our previous data for SA disposition (Yoshida et al., 2002) was used. The obtained SA and FD-4 concentrations in plasma were fitted to the two-compartment model by the nonlinear least-square method using Multi(algorithm; Damping Gauss–Newton method) (Yamaoka et al., 1981). In the elimination-phase, the amount ratio (%) of SA in skin/plasma and muscle/plasma after injection was less than 1%, and FD-4 was not detected in the skin and muscle.

### 4.3. Pharmacokinetics following i.c. injection of SA

Fig. 3 shows the time course of SA amount in each tissue after i.c. injection of SA alone (a), SA with phenylephrine (b) and SA with tolazoline (c). The drug amount in plasma was calculated using the volume of central compartment, $V_1$ (Table 1). The result for the injection of SA alone (control) was referred from our previous report (Yoshida et al., 2007). The SA amount in the muscle was under the determination limit (<0.02 μmol) at 90 min after i.c. injection. SA in the skin and muscle was also under the determination limit (<0.02 μmol) at 90 and 60 min, respectively, after i.c. injection of SA with tolazoline. The average SA amount obtained from tissues was fitted to the compartment model (Fig. 1) by the nonlinear least-square method (see Theoretical). Better agreement of observed and theoretical values was obtained by assuming that the rate constant, $k_{ms}$, from the muscle to skin was zero. Table 2 shows the calculated pharmacokinetic parameters following i.c. injection. The SA elimination rate constant from skin, $k_s$ ($k_{sm} + k_{sc}$), after i.c. injection decreased about 0.6-fold by combination with phenylephrine compared with the control group. The rate constant, $k_{sc}$, from the skin to systemic circulation of SA after i.c. injection by combination with phenylephrine was zero, and the rate constant, $k_{sm}$, from the skin to muscle increased about 2.4-fold compared with the control. On the other hand, the elimination parameter of SA from the skin, $k_s$, after i.c. injection increased about 2.1-fold by combination with tolazoline compared with the control. The constant $k_{sc}$ from the skin to systemic circulation and $k_{sm}$ from the skin to muscle of SA increased about 1.9- and 2.5-fold compared with the control, respectively.

### 4.4. Pharmacokinetics following i.c. injection of FD-4

Fig. 4 shows the time course of FD-4 in each tissue after i.c. injection of FD-4 alone (a), FD-4 with phenylephrine (b) and FD-4 with tolazoline (c). These results were analyzed by the same method as for SA disposition. Table 2 summarizes the calculated pharmacokinetic parameters following i.c. injection of FD-4. The elimination parameter of FD-4 from skin, $k_s$, decreased about 0.8-fold by combination with phenylephrine compared with the control. The $k_{sc}$ from the skin to systemic circulation decreased about 0.3-fold and $k_{sm}$ from the skin to

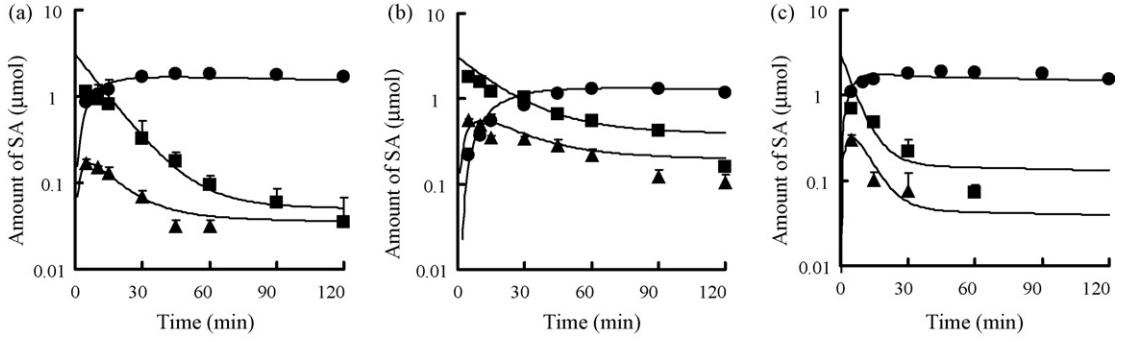


Fig. 3. Time courses of SA amount in each tissue following i.c. injection of SA–Na (3.08 μmol/20 μL) (a) combined with phenylephrine (b) and tolazoline (c). Symbols: (■), skin; (▲), muscle; (●), plasma. Each line was obtained by the curve-fitting technique. Each data point represents the mean ± S.E. of 3–12 experiments.

Table 2
Pharmacokinetic parameters of SA and FD-4 following i.c. injection

| | $k_{sc}$ (min$^{-1}$) | $k_{sm}$ (min$^{-1}$) | $k_s$ (min$^{-1}$) | $k_{mc}$(min$^{-1}$) | $k_{cs}$(min$^{-1}$) | $k_{cm}$(min$^{-1}$) |
|---|---|---|---|---|---|---|
| SA | | | | | | |
| Control[a] | $5.4 \times 10^{-2}$ | $1.9 \times 10^{-2}$ | $7.3 \times 10^{-2}$ | $2.2 \times 10^{-1}$ | $2.3 \times 10^{-3}$ | $2.7 \times 10^{-3}$ |
| +Phenylephrine | 0 | $4.5 \times 10^{-2}$ | $4.5 \times 10^{-2}$ | $1.7 \times 10^{-1}$ | $1.3 \times 10^{-2}$ | $1.2 \times 10^{-2}$ |
| +Tolazoline | $1.0 \times 10^{-1}$ | $4.8 \times 10^{-2}$ | $1.5 \times 10^{-1}$ | $2.3 \times 10^{-1}$ | $1.3 \times 10^{-2}$ | $1.8 \times 10^{-3}$ |
| FD-4 | | | | | | |
| Control | $2.7 \times 10^{-2}$ | $4.0 \times 10^{-3}$ | $3.1 \times 10^{-2}$ | $2.0 \times 10^{-1}$ | $2.4 \times 10^{-2}$ | $2.8 \times 10^{-2}$ |
| +Phenylephrine | $9.1 \times 10^{-3}$ | $1.6 \times 10^{-2}$ | $2.5 \times 10^{-2}$ | $7.3 \times 10^{-2}$ | $2.4 \times 10^{-2}$ | 0 |
| +Tolazoline | $6.0 \times 10^{-2}$ | $1.7 \times 10^{-2}$ | $7.7 \times 10^{-2}$ | $2.1 \times 10^{-1}$ | $4.6 \times 10^{-2}$ | $1.3 \times 10^{-2}$ |

$k_s$ is the sum of $k_{sm}$ and $k_{sc}$.
[a] SA parameters were referred from our previous report (Yoshida et al., 2007).

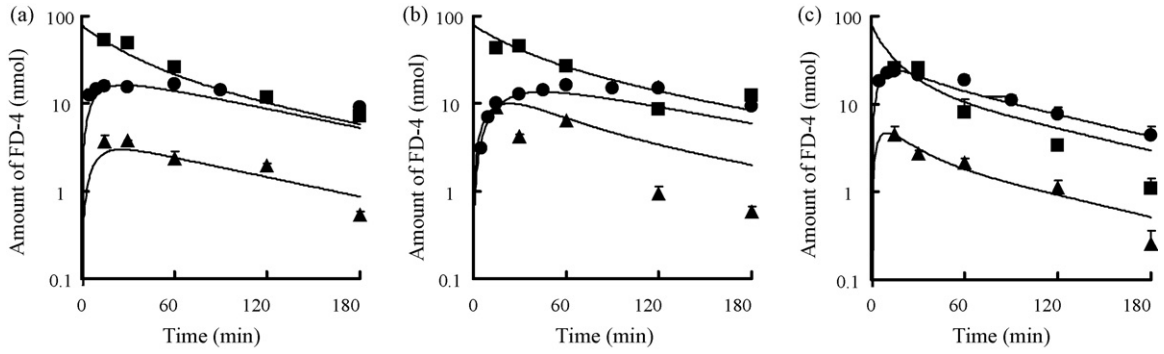


Fig. 4. Time courses of FD-4 amount in each tissue following i.c. injection (78.5 nmol/20 μL) (a) combined with phenylephrine (b) and tolazoline (c). Symbols: (■), skin; (▲), muscle; (●), plasma. Each line was obtained by the curve-fitting technique. Each data point represents the mean ± S.E. of 3–12 experiments.

muscle increased about 4.0-fold compared with the control after i.c. injection by combination with phenylephrine. In contrast, $k_s$ increased 2.5-fold by combination with tolazoline compared with the control. The $k_{sc}$ from the skin to systemic circulation and $k_{sm}$ from the skin to muscle increased about 2.2- and 4.3-fold, respectively, compared with the control.

## 5. Discussion

The present i.c. injection can be adopted for direct delivery to viable skin by evading the permeation barrier of the stratum corneum. We evaluated changes in cutaneous blood flow after i.c. injection of vasoactive agents and compared with changes in the pharmacokinetics of model drugs in the present study. The dose of vasoactive agents was adjusted to an adequate concentration in cutaneous tissues within the present experiment period. In other words, vasoconstriction was more potent by the administration of a high concentration of phenylephrine in the preliminary experiment, but the drug was not detected in plasma. Vasodilatation was more effective with the administration of a high concentration of tolazoline, but drug elimination from the injection site in skin was too fast. Since further analysis of the pharmacokinetics/pharmacodynamic relations was difficult, the doses of phenylephrine and tolazoline were set at 50 and 2.0 nmol, respectively. In addition, in the selection of model drugs other than SA and FD-4, macromolecules bigger than FD-4 were difficult to analyze pharmacokinetically due to the low-migration rate between organs and tissues.

The Laser Doppler method reflects blood flow of vasculature from the skin surface to dermal papilla (about 500 μm depth from the skin surface) (Guy et al., 1986; Tanojo et al., 1999); therefore, the change of cutaneous blood flow after i.c. injection of vasoactive agents could be observed definitely, so that the effect of phenylephrine and tolazoline on blood flow in skin was confirmed. Changes in the cutaneous blood flow may be related to the action mechanism and titer of vasoactive agents as well as their diffusivity in skin and disposition of solution. Therefore, all rate constants, $k_{xy}$ (rate constant from tissue x to y), calculated by curve-fitting in this study, are shown in the average value during the experimental period. The vasoactive agents presently loaded were too low to estimate pharmacokinetics in this experiment.

The time course of the drug amount in tissues after i.c. injection of SA and FD-4 was fitted to theoretical equations derived from the present compartment model by the nonlinear least-squares method. Since no change was observed in the elimination pharmacokinetics of the drugs after i.v. injection with and without vasoactive agents, the elimination pharmacokinetic parameters without vasoactive agents were used for the analysis of dermatopharmacokinetics and systemic drug disposition. The $k_{sc}$ and $k_{sm}$ of FD-4 after i.c. injection of FD-4 alone were smaller than the corresponding rate constants of SA. These results may be related to lower diffusivity in the skin membrane and permeability through the vascular wall of FD-4 than SA, due to differences in the molecular weight. Low migration to the systemic circulation and high migration to muscle after i.c. injection for both drugs were observed by combination

with phenylephrine. Contraction of cutaneous blood vessels in the dermis by phenylephrine may be related to suppressed drug migration from the skin to the systemic circulation, and increased drug migration to the muscle. On the other hand, the migration of drugs to the systemic circulation after i.c. injection increased by combination with tolazoline. The increase of FD-4 with/without tolazoline, 2.2-fold, was slightly higher than that of SA, 1.9-fold. Since macromolecules generally show lower permeability through blood vessels than low molecules, it is difficult for macromolecules to be absorbed into peripheral vessels. This may explain why the improvement effect on drug absorption by increased blood flow was higher in macromolecules. The $k_{sm}$ value with tolazoline was higher than that without the vasoactive agent. No evidence for the decrease in $k_{sm}$ was found by dilution of dermal vessels by coadministration of tolazoline. The reason may be predicted as follows: the drug release parameter, $k_s$ may be expressed as the sum of $k_{sc}$ and $k_{sm}$, and the value for SA as well as FD-4 became larger by the addition of tolazoline. In other words, $k_{sm}$ was estimated to be high, because increased blood flow increased the cutaneous clearance of the drugs.

## 6. Conclusions

Cutaneous blood flow changed by the addition of vasoactive agents, and this change in blood flow greatly affected the dermatopharmacokinetics (drug migration from the skin to muscle and systemic circulation) after i.c. injection. Since the permeation rate through the stratum corneum of most drugs topically applied is very slow in conventional transdermal systems, enhancement of skin permeation may be necessary to achieve adequate therapeutic effects. Since cutaneous vessel permeation must be a rate-limiting step in stratum corneum barrier-evading systems, vasoactive agents are very useful to control the transdermal delivery rate in the new transdermal delivery systems. This tendency is marked in the topical delivery system of high-molecular weight drugs. The present finding may be useful for designing transdermal delivery systems containing macromolecule drugs.

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