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Targeting of salicylate to skin and muscle following topical injections in rats

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Abstract

The process of systemic absorption and tissue targeting efficacy of salicylate (SA) following intracutaneous (i.c.), subcutaneous (s.c.) and intramuscular (i.m.) injections of its sodium salt in rats were evaluated by determining the drug concentration at the injection site and surrounding tissues. After i.c. and s.c. injections, SA was absorbed into the systemic circulation from the muscular vessels as well as the cutaneous or subcutaneous vessels beneath the injection site, and the AUC of the drug in the muscle was extremely high. Following i.m. injection, SA was rapidly absorbed into the systemic circulation mostly from the muscular vein. These results suggested that i.c. and s.c. injections have high degrees of targeting efficacy to the muscle, whereas i.m. injection is not appropriate for drug retention in muscle. In contrast, most of the topically applied drug was absorbed from the cutaneous vessels, and little drug migration to the muscle was observed. Thus, the skin pharmacokinetics of SA after i.c. injection was also markedly different from those after topical application on the skin. These results suggested that the i.c. and s.c. injections may be a good means to improve the targeting ability of drugs to the muscle as well as the skin.

Keywords: Injection; Topical application; Drug targeting; Salicylate; Remaining; Pharmacokinetic

1. Introduction

Topical formulations on skin can be classified into two types: those with systemic actions after drug uptake by cutaneous capillaries; and those with local effects in cutaneous, subcutaneous and muscular tissues (Guy and Maibach, 1983). The former has been called the transdermal therapeutic system (TTS). TTS has several advantages, i.e. maintenance of plasma concentration, avoidance of gastrointestinal first-pass effects and increase in patient compliance (Grond et al., 1997). In contrast, the latter type of formulations may efficiently deliver drugs to the local tissues with decreased side effects in other tissues. However, the barrier function of the stratum corneum generally limits transdermal delivery of most drugs. To overcome this shortcoming, various penetration enhancers and prodrugs have been utilized to

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increase the bioavailability of topically applied drugs with low skin permeability (Ogiso et al., 1995).

Recently, new techniques to improve the skin permeation of drugs such as microfabricated microneedles (Henry et al., 1998) and jet injection (Inoue et al., 1996) have been developed. These techniques are expected to produce new routes for the delivery of drugs across the skin barrier. The former has many microneedles with a length to cross the stratum corneum but not to reach the nerves in the deeper tissues. Thus, this system could be easily inserted into the skin to increase the skin permeability of drugs without pain. The latter, which is used for self-injection of insulin and growth hormone (Lindmayer et al., 1986), flushes the drug solution into the viable skin tissues through the stratum corneum using high pressure. These enhanced skin delivery systems may have similar properties to intracutaneous (i.c.), subcutaneous (s.c.) or intramuscular (i.m.) injections. Drug disposition at the sites of injection and diseased tissues usually must be related to the therapeutic effect. Although the systemic absorption of drugs after s.c. and i.m. injections has been evaluated, there have been only a few reports on drug disposition in the skin and surrounding tissue (Kakemi et al., 1969). The i.c. injection study was carried out to observe antigen–antibody reactions, e.g. the tuberculin test (Olivier, 2000), but has not been established yet for therapeutic means.

In the present study, the targeting and retention ability of a model drug, salicylate (SA), to skin and muscle were evaluated following i.c., s.c. or i.m. injections of its sodium salt (SA-Na) in rats, and the results were compared with those obtained after topical application on the skin.

2. Materials and methods

2.1. Materials

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

2.2. Animals

Male Wistar rats, 300 ± 20 g, supplied by Ishikawa Experimental Animal laboratory (Fukaya, Saitama, Japan) were used. 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 guidance of Life Science Research Center, Josai University.

2.3. Preparation of cutaneous blood flow-stopped rats

To clarify the drug migration process in skin, cutaneous blood flow-stopped rats were prepared. The abdominal skin was excised (3 cm in diameter) from rats, and then the skin was reattached to the same position. The incised region was sutured with Michel's clamp.

2.4. Intravenous injection study

Intravenous (i.v.) injection study was conducted to obtain the elimination pharmacokinetic parameters and distribution characteristics of SA to the local region in rats. SA-Na (39.02–187.0 μmol/kg) was injected into the jugular vein, and blood samples were collected from the contralateral jugular vein at predetermined times. The resulting samples were centrifuged to obtain 100 μl of plasma. Skin and muscle (3 cm in diameter) were excised at the end of the experiment from the same position in cases of i.c., s.c. and i.m. injections and topical application. The skin contained the subcutaneous tissue. The samples were frozen and stored at -20 °C until determination. In the present study, the amounts of SA in skin and muscle are indicated as those within a circle 3 cm in diameter, and the results are expressed as percentage of injected dose (%ID).

2.5. I.c., s.c. and i.m. injection studies

SA-Na (62.46 μmol/20 μl in water) was loaded into the abdominal site in anesthetized rats by i.c.,

s.c. and i.m. injections with a 27G needle. In the case of i.m. injection, the tip of the needle was inserted at a depth of 2 mm from the skin surface. Plasma, skin and muscle samples were obtained as described above. The i.c. injection study was carried out separately in the cutaneous blood flow-stopped rats.

2.6. Topical application study

A glass diffusion cell (effective diffusion area, 3.14 cm^2) was attached to the stratum corneum-stripped skin (Washitake et al., 1973) on the rat abdomen using cyanoacrylate adhesive (Aron Alpha®, Konishi, Osaka, Japan). SA-Na aqueous solution at a concentration of 1.0% (2.0 ml) was applied to the diffusion cell, and this drug solution was changed every 1 h to avoid a marked decrease in the SA activity on the skin. Plasma, skin and muscle samples were obtained as described above. Topical application was also carried out in the cutaneous blood flow-stopped rats.

2.7. SA assay

Each plasma sample was mixed with two volumes of acetonitrile containing propyl *p*-hydroxybenzoate as an internal standard, and centrifuged at 13 600 rpm for 5 min at 5 °C. Excised skin and muscle were homogenized with 4 ml each of physiological saline and acetonitrile containing the internal standard, and centrifuged to obtain the supernatant. The resulting samples were injected into an HPLC column. The HPLC system consisted of a pump (LC-10AS Shimadzu, Kyoto, Japan), an 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 a reverse-phase column (LiChrospher® 100, RP-18e (5 μm) 250-4, Merck, Darmstadt, 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 the 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.

2.8. Pharmacokinetic analysis

Fig. 1 shows the compartment models of SA following different routes of administration. The model for i.c. and s.c. injections consisted of skin, muscle, central and peripheral compartments (Fig. 1a). It is important to consider the size of depot (administration site) after the injection of drugs. In the model for i.m. injection (Fig. 1b), the muscle compartment was mathematically divided into the administration site and the surrounding area. The administration site of the muscle layer (3 cm in diameter) was extracted to determine the SA level for evaluating the drug disposition in the injection site after the i.m. injection. The volume of the muscle must be $(3\ cm/2)^2 \times \pi \times L$, where L is thickness of the muscle layer. Consequently, the muscle and systemic dis-

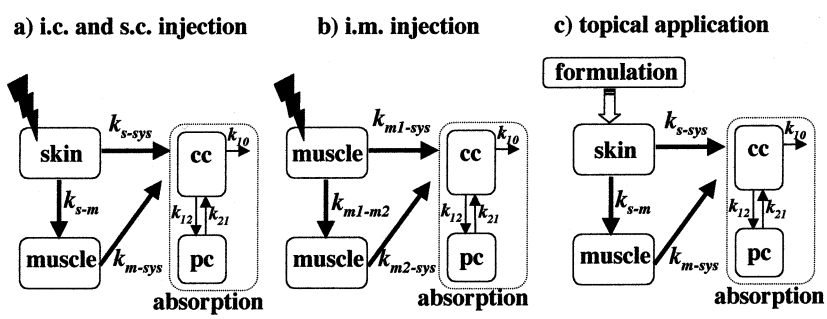

Fig. 1. Pharmacokinetic model for SA following (a) i.c. and s.c injections; (b) i.m. injection; and (c) topical application. Abbreviations: cc, central compartment; and pc, peripheral compartment.

positions of SA were well expressed by a pharmacokinetic model having two muscle compartments for the injection site (muscle) rather than a single compartment for the muscle. It was confirmed in a preliminary experiment that SA rapidly diffused from the i.m. injection site into the surrounding muscle. In cases of pharmacokinetic analysis for i.c. and s.c. injections, however, single muscle-compartment model was more convenient than the two muscles-compartment model from the results obtained. In topical application (Fig. 1c), drug migration rate from the formulation to the skin was assumed to be of the zero order, since the drug concentration in the donor solution was almost constant due to periodical replacement with fresh solution. Linear 2-compartment model was assumed from elimination pharmacokinetics of SA (see Section 3). Absorption compartment was defined, as shown in Fig. 1a–c, for ease of data treatment using the volume of distribution of the central compartment, V_1 and the rate constants, k_{12}, k_{21} and k_{10}. In the analysis of drug disposition for topical application, the drug amount in the skin and the muscle were deducted by the amount of redistribution from the systemic circulation (the ratio of drug amount in tissue and plasma) obtained after i.v. injection, although the amount of redistribution was negligible in i.c., s.c. and i.m. injection studies. The V_1, k_{12}, k_{21} and k_{10} were obtained by curve-fitting to plasma concentration data after the i.v. injection. The absorption amount of SA at time t can be represented as the sum of $C_t \times V_1$, $X_{pc,t}$ and $AUC_{0-t} \times k_1 \times V_1$ (C_t and $X_{pc,t}$: plasma concentration and amount in peripheral compartment of SA at time t). $X_{pc,t}$ was obtained by convolution using the elimination pharmacokinetic parameters and plasma concentration data of SA. Since the sum of the amount in each compartment and the absorption amount was approximately consistent with the injected dose, as determined in a preliminary experiment, the sum was considered as the dose. The obtained SA concentration or amounts in plasma and tissues were fitted to the compartment model by the nonlinear least-square method (Yamaoka et al., 1981).

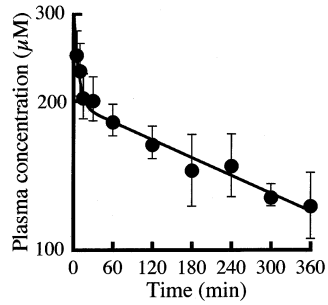

Fig. 2. Time courses of changes in plasma concentration of SA following the i.v. injection of SA-Na at a dose of 39.02 μmol/kg. Each data point represents the mean ± SE of three experiments.

3. Results

3.1. Intravenous injection study

SA-Na was intravenously injected at doses of 39.02–187.0 μmol/kg in rats. Fig. 2 shows the typical time course of changes in the plasma concentration of SA administered at a dose of 39.02 μmol/kg. The elimination pharmacokinetics of SA was obeyed to the linear 2-compartment model at all doses tested, which was confirmed by almost the same total body clearance. Table 1 summarizes the pharmacokinetic parameters and T/P (tissue amount/plasma amount) ratio of SA at 60 min. The ratio was used for the analysis of distribution fraction of SA from the application site or the systemic circulation to the tissue.

Table 1
Pharmacokinetic parameters and tissue distribution ratio following i.v. injection of SA-Na (39.02 μmol/kg) in rats

V_1 (ml/kg)	124.33 ± 7.08
k_{12} (/min)	$(6.26 \pm 0.34) \times 10^{-2}$
k_{21} (/min)	$(9.28 \pm 0.34) \times 10^{-2}$
k_{10} (/min)	$(2.21 \pm 0.32) \times 10^{-3}$
Skin–plasma ratio [a]	$(5.58 \pm 0.39) \times 10^{-3}$
Muscle–plasma ratio [b]	$(8.17 \pm 0.62) \times 10^{-3}$

Each value represents the mean ± SE of three experiments.

[a] SA amount in skin (μmol/kg)/(plasma concentration of SA (μmol/ml) × V_1 (ml/kg)) at 60 min.

[b] SA amount in muscle (μmol/kg)/(plasma concentration of SA (μmol/ml) × V_1 (ml/kg)) at 60 min.

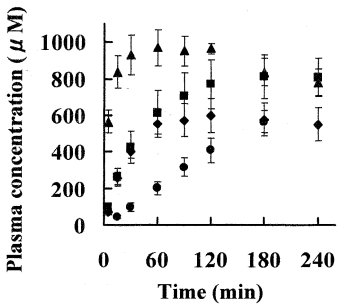

Fig. 3. Time courses of changes in plasma concentration of SA following i.c., s.c. and i.m. injections (62.46 μmol SA-Na/20 μl) and topical application (1% SA-Na, 2 ml). Symbols: i.c. injection (◆); s.c. injection (■); i.m. injection (▲); topical application (●). Each data point represents the mean ± SE of 3–7 experiments.

3.2. I.c., s.c. and i.m. injections and topical application studies

Fig. 3 shows the time courses of changes in the plasma concentration of SA after i.c., s.c. and i.m. injections and topical application. Following i.m. injection, a more rapid absorption of SA was observed from the injection site to the systemic circulation than following i.c. and s.c. injections. This observation was related to differences in blood flow in the skin and muscle (Gu et al., 1999). In topical application, plasma concentration of SA gradually increased during the experimental period.

Fig. 4 shows the time courses of changes in the SA amount (%ID) in each compartment following i.c., s.c. and i.m. injections and topical application. Following i.c. and s.c. injections (Fig. 4a and b), the amount of SA distributed from the systemic circulation to the skin and muscle, calculated from the T/P ratio, seemed to be negligible as compared with the total amount in the skin or muscle. On the other hand, following i.m. injection (Fig. 4c) a rapid decrease in the amount of SA in the muscle and no direct migration of SA from the muscle to the skin were observed. Most of the drug in the skin was caused by drug migration from the systemic circulation. In topical application (Fig. 4d), the amounts of SA distributed in the skin and muscle were the majority of the total amount that penetrated across the skin barrier. Thus, the SA amounts in the skin and muscle are expressed as the difference between the total amount in the tissues and the amount distributed from the systemic circulation (Fig. 4d). This difference was calculated by the T/P ratio. Following i.c. and s.c. injections, the remaining amount of SA in the muscle beneath the site of injection was prolonged compared with that following the i.m. injection.

These experimental data were fitted to a simple compartment model. Lines in Fig. 4 show the simultaneously fitting ones to drug levels in skin, muscle and absorption amount. Fitting line for the skin amount and the amount absorbed were a little higher and lower, respectively, than the observed ones at the early stage after i.c. and s.c. injections. This observation suggested that the first-order kinetics for the drug migration from the injection site to the systemic circulation was too simple to express such a complex migration process of SA. However, we judged that this simple pharmacokinetic model is enough to elucidate the difference of skin dispositions of SA after i.c., s.c. and i.m. injections and topical application. The rate constants obtained using this curve-fitting are summarized in Table 2. These constants

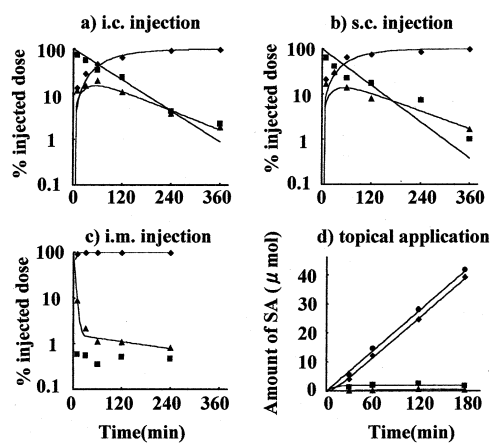

Fig. 4. Time courses of changes in %ID of SA following (a) i.c., (b) s.c., and (c) i.m. injections of SA-Na (62.46 μmol/20 μl); and (d) the topical application of SA-Na (1% SA-Na, 2 ml). Symbols: skin (■); muscle (▲); absorption amount (◆); total mount of skin permeation (●). The line was obtained by curve-fitting. Each data point represents the mean of 3–4 experiments.

Table 2
Pharmacokinetic parameters following i.c., s.c., i.m. injection (62.46 μmol/20 μl) and topical application (1% SA-Na, 2 ml/3.14 cm^2) of SA-Na

	k_{s-m}	k_{s-sys}	k_{m-sys}	k_s
i.c. injection	8.24×10^{-3}	4.68×10^{-3}	3.41×10^{-2}	1.31×10^{-2}
s.c. injection	7.58×10^{-3}	7.92×10^{-3}	3.68×10^{-2}	1.55×10^{-2}
Topical application	4.95×10^{-3}	1.10×10^{-1}	1.52×10^{-2}	1.15×10^{-1}
	k_{m1-m2}	k_{m1-sys}	k_{m2-sys}	k_m
I.m. injection	2.94×10^{-3}	2.51×10^{-1}	1.90×10^{-1}	2.54×10^{-1}

k_{s-m}, k_{s-sys}, k_{m-sys} and k_s are the first-order rate constants from skin to muscle, from skin to systemic circulation, from muscle to systemic circulation and sum of k_{s-m} and k_{s-sys}, respectively. k_{m1-m2}, k_{m1-sys}, k_{m2-sys}, and k_m are the first-order rate constants from muscle at the injected site to the surrounding muscle, from muscle at the injected site to systemic circulation, from muscle to systemic circulation and elimination from muscle at the injected site, respectively. Each value was obtained by curve-fitting using average values from three experiments.

were expressed as the average in the experiment duration. Thus, a small deviance was caused from data points. The majority of drug distributed from the topical formulation to the skin is taken up by the cutaneous vessels and transferred to the systemic circulation (Yanagimoto et al., 1999). In this study, the rate constant obtained following topical application was consistent with that reported earlier. The first-order rate constant of migration from the skin to the systemic circulation, k_{s-sys} was much greater than that from the skin to the muscle, k_{s-m}. On the other hand, k_{s-sys} was lower and k_{s-m} was higher following i.c. and s.c. injections than following topical application, indicating that the direct migration ratio of the drug from skin to muscle was increased by i.c. and s.c. injections. The transfer rate constants of SA from the drug loaded sites, k_s (i.e. $k_{s-sys}+k_{s-m}$) following i.c. and s.c. injections were lower than that following topical application. Following i.m. injection, the most rapid clearance of SA from the injection site was observed with a high rate constant, k_m (i.e. $k_{m1-m2}+k_{m1-sys}$).

3.3. I.c. injection and topical application studies using cutaneous blood flow-stopped rats

To compare the drug migration rate from the skin into the systemic circulation or deeper tissue, SA level in each compartment was determined following i.c. injection and topical application in the cutaneous blood flow-stopped rats. Elimination of the drug from cutaneous vessels was assumed negligible in this experimental model. Fig. 5 compares the amounts of SA in each compartment 60 and 120 min after i.c. injection and topical application in the intact and cutaneous blood flow-stopped rats. The residual amount in skin and the total absorption amount of SA in the pretreated rats were increased and decreased, respectively, compared with those in intact rats

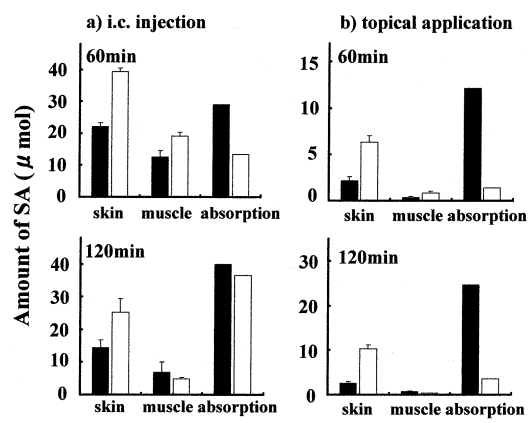

Fig. 5. SA amount in each compartment 60 and 120 min following (a) i.c. injection of SA-Na (62.46 μmol/20 μl) and (b) topical application of SA-Na (1% SA-Na, 2 ml) in intact rats (closed columns) and cutaneous blood flow-stopped rats (open columns). Each column for skin and muscle represents the mean ± SE of 4–5 experiments and that for absorption represents the mean of 4–5 experiments.

following i.c. injection and topical application, but the ratio of change following topical application was larger than that following i.c. injection. These results suggested that the cutaneous clearance was predominant for drug migration from skin as a drug-loaded site after topical application. On the other hand, i.c. injection increased the direct drug migration from skin to muscle.

4. Discussion

Rapid elimination of SA from the muscle and the early appearance in the systemic circulation were observed after i.m. injection. No direct migration of SA from muscle to skin, located below the site of administration was observed. These results suggested that i.m. injection is not appropriate for the local targeting of drugs. Prolonged retention of SA in the skin as well as in the underlying muscle was found after i.c. and s.c. injections, which was probably due to the slow transfer of SA from the administration site to the systemic circulation. These observations suggested that i.c. injection may be utilized to improve drug targeting to skin and muscle, while maintaining an effective drug concentration. Little direct drug migration from skin to muscle was found following topical application, because most of the drug distributed to the skin is absorbed by the systemic circulation through the cutaneous vessels.

The direct transport of SA to the muscle was evaluated by i.c. injection and topical application in the cutaneous blood flow-stopped rats. Following topical application, most of the drug in the muscle was due to direct migration from the skin, and not from the systemic circulation (Yanagimoto et al., 1999). The present results regarding topical application agreed well with those reported previously. Correspondingly, most of the SA in the muscle following i.c. and s.c. injections was due to direct delivery from the injection site.

In this study, hypertonic SA solution (62.46 μmol/20 μl of SA-Na in water) was injected. An increased rate of absorption into the systemic circulation was reported after the i.m. injection of hypertonic solution compared with that after the i.m. injection of isotonic solution (Kakemi et al., 1971). The damage and pain caused by the injectant at the injected site are clinical problems. In fact, hypertonic solution caused edema at the site of injection in a time-dependent manner. The osmotic pressure of the injectant may have an effect on the isotonicity in the biological fluid and tissues. This change may be a nonlinear phenomenon, dependent on time. This assumption probably relates to the difference between the fitting value and the observed ones in the skin amount and the amount of SA absorbed during the experimental period after i.c. and s.c. injections (Fig. 4a and b). Additionally, the differences in the residual amount in skin and the absorption amount of SA between intact and pretreated rats after i.c. injection at 60 min were larger than those at 120 min (Fig. 5a). Therefore, the rate constants obtained calculated by curve-fitting in this experiment, k, may have been the average values during the experiment. To obtain correct values, it is necessary to evaluate the effects of osmotic pressure of the injectant. In addition, the volume and dose of injectant likely affect pharmacokinetic properties at the injection site. However, the present results can be utilized for the development of DDS with increased local targeting efficacy. The k values obtained, even if they were averages, were sufficiently adaptive to allow comparisons between experiments. Further studies of the effects of the osmotic pressure, volume and dose of injectant for i.c. and s.c. injections are currently in progress in our laboratories and will be reported in the near future.

5. Conclusions

Skin disposition of SA was greatly influenced by route and means of administration. Drug distributed on or after topical formulation to skin is mainly absorbed into the systemic circulation through the cutaneous vessels. On the other hand, drug loaded by i.c. and s.c. injections migrates into the muscle as well as the systemic circulation. Thus, i.c. and s.c. injections can be utilized to improve drug targeting to skin and muscle while maintaining the effective drug concentration, which may result in better therapeutic effects.

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