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Enhancement of topical delivery of drugs via direct penetration by reducing blood flow rate in skin

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Abstract

The purpose of this work was to investigate the effect of blood flow in the skin on the direct penetration of topically applied drugs into the muscular layer, and to show that the skin blood flow could also be one of the important factors determining the direct penetration of drugs to the muscular layer. In vivo percutaneous absorption study was performed for antipyrine, salicylic acid or diclofenac by using rats with tape-stripped skin. Phenylephrine, which is well known to reduce the local blood flow by vasoconstrictor action, was topically applied to decrease the local blood flow in the skin. The concentrations of drugs in viable skin and muscle, and the local blood flow in the skin under the applied and the contralateral sites were determined to evaluate the effect of the local blood flow on the delivery of topically applied drugs into the muscular layer. Dose dependency for the effect of phenylephrine was, first of all, investigated for antipyrine in the range from 0.4 to 10 μmol. The distribution of antipyrine into the viable skin and muscular layer 2 h after topical application significantly increased, but the effect of phenylephrine was saturated around 2 μmol and the dose-dependent profiles for both tissues were almost superimposed. On the other hand, the fraction dose absorbed, plasma concentration and concentrations in viable skin and muscular layer under the contralateral site showed the decreasing tendency and the saturation of the effect around 2 μmol. To confirm the effect of phenylephrine on the local blood flow in the skin, the skin blood flow was measured 2 h after topical application of 2 μmol phenylephrine, and the significant decrease in the blood flow was recognized. In vivo percutaneous absorption studies were performed for salicylic acid and diclofenac, too. Extensive enhancement of penetration into the viable skin and muscular layer was observed for both drugs, although total absorption from the donor cell showed the decreasing tendency. In conclusion, direct penetration of drugs applied topically is enhanced by reducing the local blood flow in the skin, which would be a possible approach to improve the local delivery of drugs applied topically.

Keywords: Direct penetration; Skin blood flow; Local therapeutics; Transdermal absorption; Topical application

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1. Introduction

Transdermal delivery system for the local therapeutics would be expected to avoid the systemic absorption and to make the substantial penetration into deeper tissues, such as muscle, of topically applied drugs as much as possible. As a main barrier against transdermal absorption, the stratum corneum, could be overcome by a lot of promising physical and/or chemical approaches (Barry, 2001; Higaki et al., 2003), the regulation of intradermal disposition of drugs after the passage across the stratum corneum would be the next issue to be pursued to develop the efficient transdermal delivery system. However, there is still controversy regarding with the mechanisms by which topically applied drugs are distributed into deeper tissues, i.e. "direct penetration" or "re-distribution from blood flow after systemic absorption". Diclofenac (Radermacher et al., 1991) and felbinac (Dawson et al., 1988) were reported to distribute into deeper tissues mainly from systemic blood supply. On the other hand, the substantial direct penetration was shown for estradiol, progesterone (Marty et al., 1989), salicylic acid (Singh and Roberts, 1993; Cross et al., 1997) and piroxicam (M-Riviere et al., 1993). In the previous studies, we performed the in vivo transdermal absorption studies using rats with tape-stripped skin for seven different drugs including diclofenac, felbinac and salicylic acid, and clearly showed that these topically applied drugs are substantially distributed into muscular layer via direct penetration (Nakayama et al., 1999; Higaki et al., 2002). The pharmacokinetic analysis based on the six-compartment model with contralateral tissues explicitly indicated that the contribution of direct penetration to the deeper tissue distribution of drugs applied topically is dependent on drugs, and that the balance in the contribution between direct penetration and blood supply is time-dependently changed (Nakayama et al., 1999; Higaki et al., 2002). Furthermore, we showed that the unbound fraction of drugs in the viable skin is possibly one of the most important factors to regulate the direct penetration of drugs into the muscular layer (Higaki et al., 2002). Blood flow in the skin, particularly the dermis, must also be an important factor, because the blood vessels in the dermis are supposed to absorb and dilute most compounds passing the epidermis, keeping a "sink" condition and thus promoting the percutaneous absorption (Barry, 2002). However, at the same time, this means that blood flow in the dermis prevents drugs from directly penetrating into deeper tissues by removing them to the systemic circulation. It was also suggested that topical penetration of drugs could be dependent on the distribution of the local cutaneous vasculature (McNeill et al., 1992; M-Riviere et al., 1993). In the present study, therefore, we investigated the effect of the skin blood flow on the penetration of drugs into the muscular layer after their topical application, by reducing the skin blood flow rate with topical application of phenylephrine, α_1-agonist, in rats with stripped skin.

2. Materials and methods

2.1. Materials

Diclofenac sodium, aminopyrine and L-phenylephrine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Antipyrine and salicylic acid were obtained from Ishizu Pharmaceutical Co. (Osaka, Japan). Flufenamic acid and *o*-anisic acid were obtained from Sankyo (Tokyo) and Tokyo Chemical Industry Co. (Tokyo), respectively. All other reagents were of the highest grade commercially available.

2.2. Animals

Male Wistar rats (Japan SLC, Hamamatsu, Japan), maintained at 25 °C and 55% of humidity, were allowed free access to standard laboratory chow (Clea Japan, Tokyo) and water prior to the experiments. Rats weighing 230–270 g were randomly assigned to each experimental group. Our investigations were performed after approval by our local ethical committee at Okayama University and in accordance with "Principles of Laboratory Animal Care (NIH publication #85-23)".

2.3. In vivo transdermal absorption study

Abdominal hair was removed by using 7% thioglycolic acid gel 2 days before performing the absorption study and the stratum corneum was stripped with adhesive tape about twenty times under urethane anesthesia just before starting the absorption study. Under urethane anesthesia, 2 ml of a model drug solution

(5 mM) with or without phenylephrine was applied to the donor cell, of which the effective area is 4.91 cm^2, attached on the stripped abdominal skin with Aron Alpha (Toa Chemicals Co. Ltd., Tokyo). After rats were sacrificed at 2 h, concentrations of the drug in the donor cell, viable skin, muscle, contralateral skin, contralateral muscle and plasma were determined. Fraction dose absorbed was calculated by subtracting a remaining amount of drug in donor cell from an initial dose of drug.

2.4. Measurement of local blood flow rate in rat skin

The local blood flow in the skin was non-invasively measured based on Doppler principle by PeriScan PIM II Laser Doppler perfusion imager (Permed AB, Järfälla, Sweden) (Nilsson, 1984; Ahn et al., 1987) 2 h after the topical application of phenylephrine (2 μmol) on the stripped abdominal skin in vivo.

2.5. Determination of model drugs

2.5.1. In plasma

Plasma was deproteinized with acetonitrile after the addition of internal standard. As an internal standard, flufenamic acid, aminopyrine and *o*-anisic acid were used for diclofenac, antipyrine and salicylic acid, respectively. An aliquot of the supernatant was introduced onto HPLC system described later. In the case of drugs in the donor cell, an aliquot of the solution in the donor cell was just injected onto HPLC system.

2.5.2. In viable skin and muscle

Viable skin and muscle were excised and each of them was placed into the centrifuging tube, where an internal standard and 1.5 ml of 0.5 N NaOH were added and the tissues were solubilized in the boiling water bath for 30 min. For antipyrine, the mixtures were extracted with dichloromethane. In the case of diclofenac or salicylic acid, diethylether or hexane was used for the extraction, respectively, and the aqueous phase was extracted with chloroform after adding 3 N HCl. No substantial degradation of drugs was recognized during these processes. The residue obtained by evaporating the organic solvent phase was dissolved in the mobile phase used in HPLC analysis and injected onto HPLC system. HPLC system consisted of a model LC-6A HPLC pump (Shimadzu, Kyoto, Japan) and a UV detector (SPD-6A; Shimadzu) set at 254, 280 and 230 nm for antipyrine, diclofenac and salicylic acid, respectively, or a fluorescence detector (RF-535; Shimadzu) set at 270 nm and 305 nm of excitation and emission wavelengths, respectively, for phenylephrine. Analytical column was Inertsil $5C_{18}$ (150 × 4.6 mm i.d., GL Sciences, Tokyo). The mobile phase was delivered at 1 ml/min and the composition was as follows: 20 mM phosphate buffer (pH 7.4)–methanol (65:35, v/v for antipyrine); 22 mM acetate buffer (pH 7.4)–methanol–acetonitrile (50:25:25, v/v for diclofenac); 0.5% phosphoric acid–acetonitrile (75:25, v/v for salicylic acid); 1% acetic acid–methanol (90:10, v/v for phenylephrine). For quantitative calculations, a Shimadzu C-R6A data module was employed. Drug concentrations in plasma and tissues were calculated based on standard curves. The coefficient of variation (CV) for each standard curve ranged from 0.1 to 13.0% and the squared correlation coefficient was over 0.999 for all the drugs examined in the present study.

2.6. Statistical analysis

Results are expressed as the mean ± S.E. of three or more experiments. Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Dunnet's method or Student's *t*-test.

3. Results and discussion

Phenylephrine was chosen to reduce the local blood flow in the skin, because this agent is an α_1-receptor selective agonist and causes the peripheral vasoconstriction (Hoffman and Lefkowitz, 1995). Furthermore, phenylephrine decreased the systemic absorption (Urtti and Kyyronen, 1989; Kyyronen and Urtti, 1990) and improved the local effect of drugs applied topically by the peripheral vasoconstriction (Urtti and Kyyronen, 1989; Millay et al., 1991; Catterall and Mackie, 1995). Therefore, the effect of phenylephrine on the penetration of topically applied drugs into viable skin and muscular layer was examined. In Fig. 1, the distribution of antipyrine was determined 2 h after

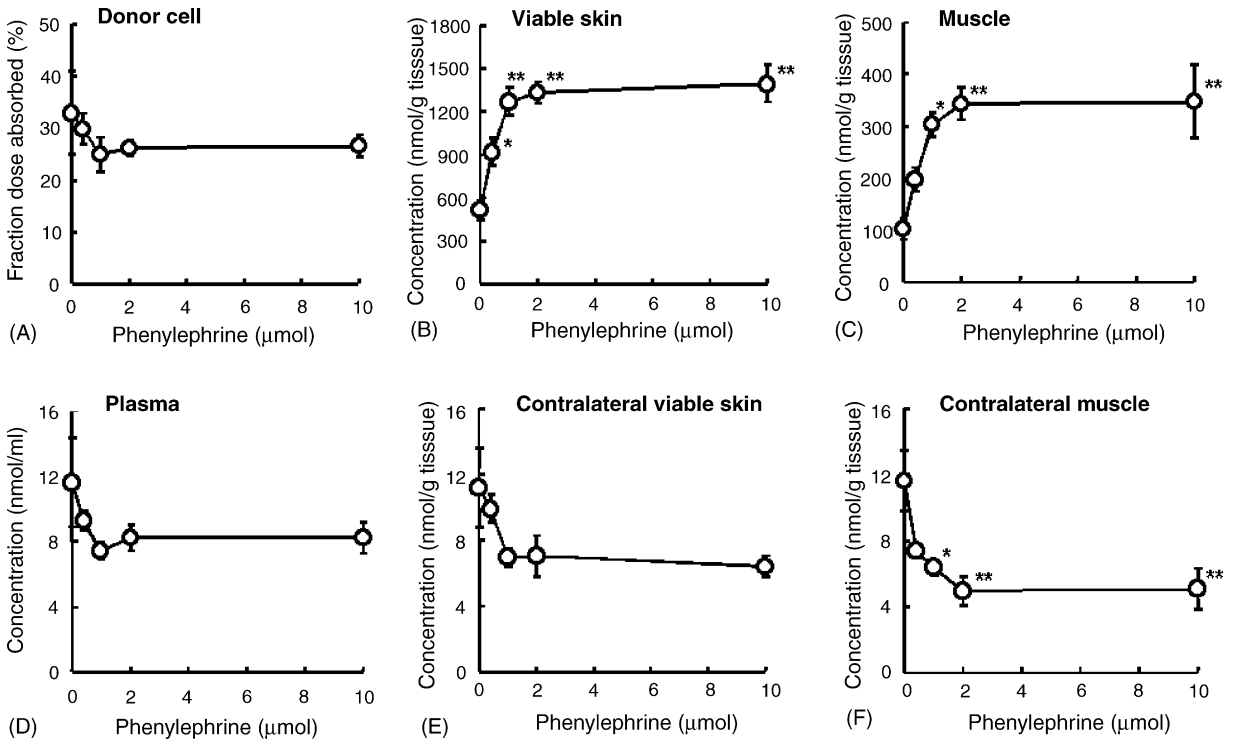

Fig. 1. Effect of phenylephrine concentration on dermal absorption of antipyrine after topical application, Antipyrine was determined 2 h after its topical application with phenylephrine. Results are expressed as the mean with a vertical bar showing S.E. value of at least three experiments. $^{**}p < 0.01$; $^{*}p < 0.05$, compared with control (phenylephrine 0 mM).

the topical application with phenylephrine ranged from 0.4 to 10 μmol. As reported previously (Nakayama et al., 1999), t_{max} for viable skin and muscle was 2 h for antipyrine and the concentrations from 2 to 4 h were very similar in plasma, contralateral viable skin or contralateral muscle. Therefore, the time period for determination was fixed at 2 h after topical application. The concentrations of antipyrine in viable skin and muscle beneath the application site were extensively higher than those at contralateral site, which were comparable with the plasma concentrations. This result explicitly indicates that the direct penetration is extremely predominant for the distribution of antipyrine into the muscular layer, and it is quite reasonable, considering our previous reports that the contribution of direct penetration to muscular distribution accounts for around 90% for 10 h (Nakayama et al., 1999; Higaki et al., 2002) and 95% at 2 h (Nakayama et al., 1999) after topical application of antipyrine. Phenylephrine significantly increased the direct penetration of antipyrine to the viable skin and the muscular layer by a dose-dependent manner. On the other hand, the fraction dose absorbed tended to decrease by phenylephrine and the similar dose-dependent profiles were observed in plasma concentrations, the concentrations in viable skin and muscle under contralateral site. These results clearly reveal that the systemic absorption after topical application was decreased by the local vasoconstriction in the skin, resulting in the decrease of the distribution into the viable skin and muscle via re-distribution from the blood supply. As total absorption tended to decrease, the systemic absorption would still be predominant comparing with the local penetration after topical application. However, the direct penetration was extensively increased by the co-application with phenylephrine. As the effect of phenylephrine was saturated around 2 μmol as shown in Fig. 1, we measured the local blood flow in the skin based on Doppler principle and confirmed the significant decrease in the skin blood flow by 2 μmol phenylephrine (Table 1). No change in the

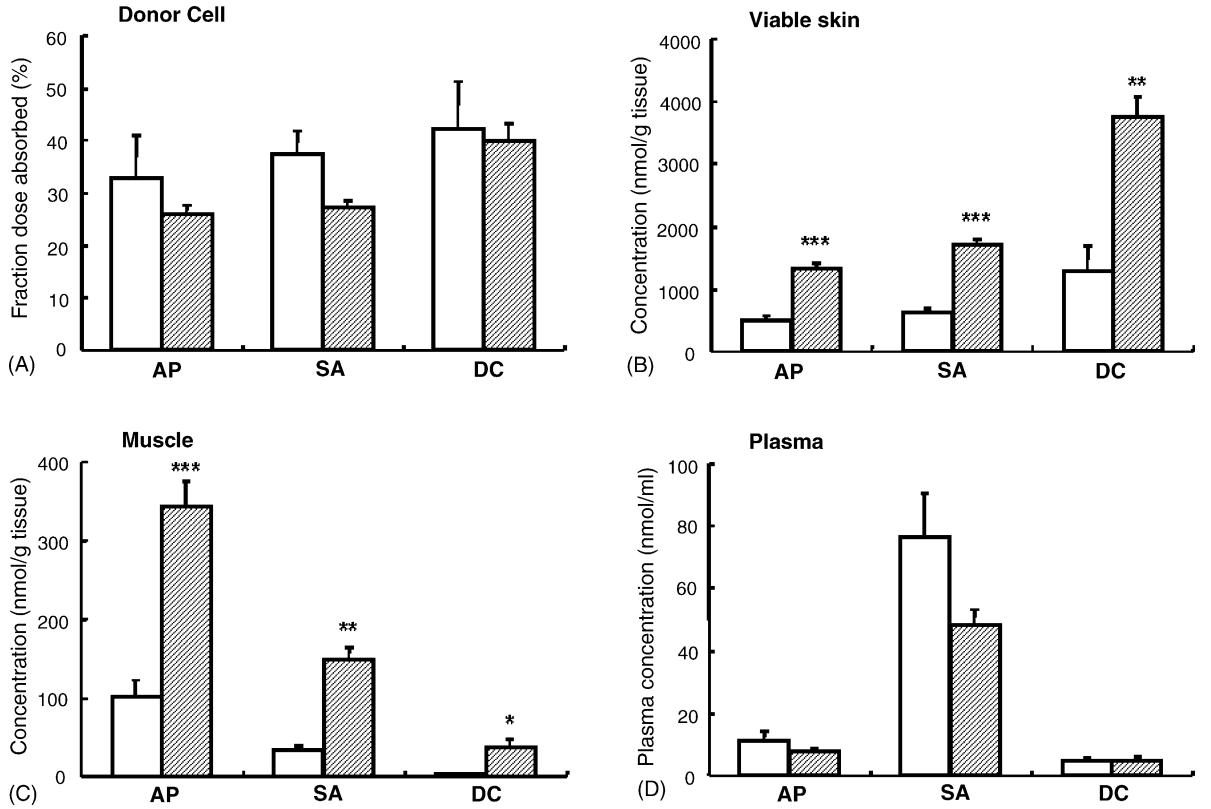

Fig. 2. Enhanced drug penetration by phenylephrine after topical application, AP, SA and DC represent antipyrine, salicylic acid and diclofenac, respectively. Results are expressed as the mean with a vertical bar showing S.E. value of at least three experiments. Key: □, control; ▨, 2 μmol phenylephrine. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$, compared with control.

blood flow of the stripped skin and intact skin below the contralateral site was observed, indicating that the vasoconstriction by phenylephrine was just a local effect for the skin under the applied site. Then, 2 μmol phenylephrine was employed to examine the local distribution at 2 h for salicylic acid and diclofenac, which are different from antipyrine in the intradermal disposition kinetics, but the direct penetration is also predominant for their muscular distribution (diclofenac, 79%; salicylic acid, 72%) (Higaki et al., 2002). The results are summarized with those for antipyrine in Fig. 2. The distribution to viable skin and muscle was significantly enhanced for salicylic acid and diclofenac as was observed for antipyrine. Although total absorption and plasma concentration of diclofenac was not significantly changed, the concentration in viable skin and muscle was extensively enhanced. As the systemic absorption would be overwhelming for diclofenac after topical application, the little decreasing tendency in the systemic absorption might yield the large increase in the direct penetration. As reported in the previous study (Higaki et al., 2002), transdermal absorption kinetics

Table 1
Effect of phenylephrine on skin blood flow rate after its topical application in rats

Experimental condition	Skin blood flow rate (V)		
	Applied site	Contralateral site	
	Stripped skin	Stripped skin	Intact skin
Control	1.45 ± 0.10	1.59 ± 0.06	2.31 ± 0.32
+ phenylephrine	0.94 ± 0.01*	1.63 ± 0.28	2.20 ± 0.14

Skin blood flow rate was non-invasively measured with PeriScan PIM II perfusion imager 2 h after the topical application of phenylephrine (2 μmol) on the stripped abdominal skin in vivo. Unit for skin blood flow rate is voltage (V). 29.10 ± 1.08% of phenylephrine was absorbed in 2 h. *$p < 0.02$ compared with control.

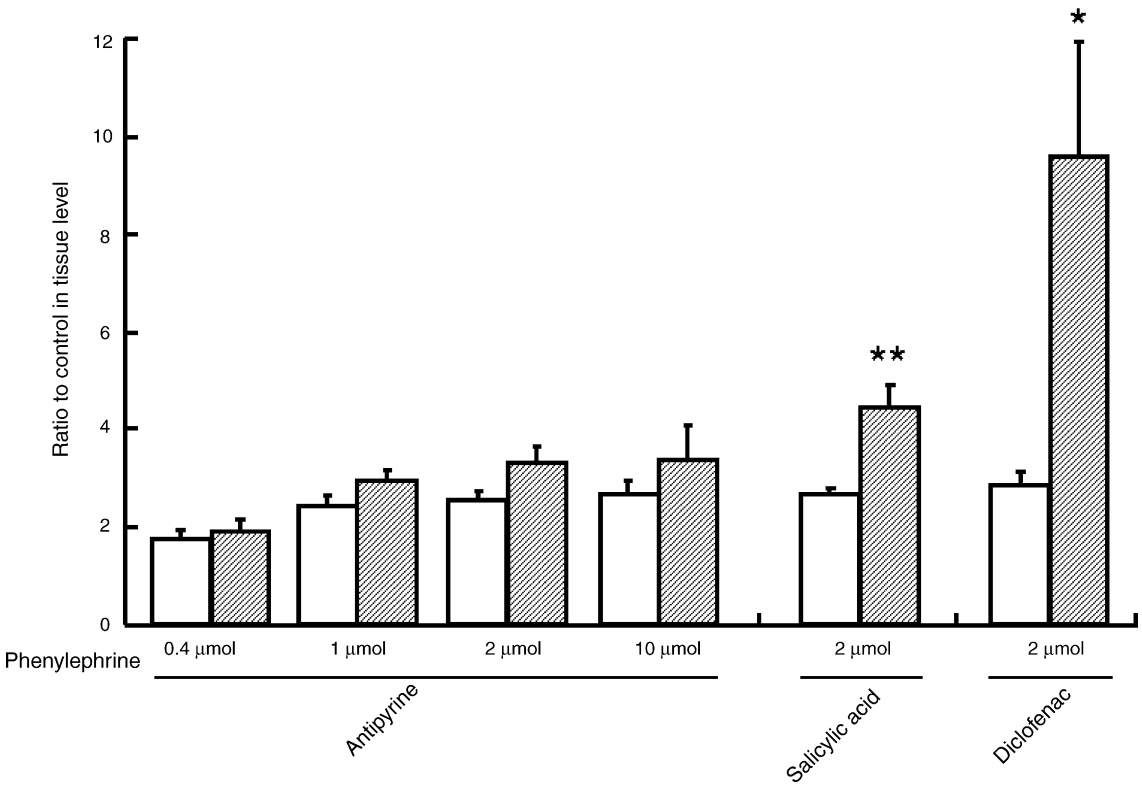

Fig. 3. Enhancement ratio in local distribution into viable skin and muscular layer by phenylephrine, Results are expressed as the mean with a vertical bar showing S.E. value of at least three experiments. "Ratio to control in tissue level" means the ratio of tissue level of drug in phenylephrine-treated rats to that in control rats. Key: □, the viable skin; ▨, the muscle. $^{**}p < 0.01$; $^{*}p < 0.05$, compared with the viable skin.

for salicylic acid and diclofenac has already been evaluated. The maximal concentrations of these drugs in viable skin and muscle under the application site are as follows: salicylic acid; viable skin 810 ± 110 nmol/g tissue at 1 h, muscle 51 ± 10 nmol/g tissue at 3 h: diclofenac; viable skin 1310 ± 373 nmol/g tissue at 2 h, muscle 5.4 ± 2.3 nmol/g tissue at 3 h. Therefore, the present results evidently show the enhancement of the direct penetration to viable skin and muscle under the application site for the two compounds as well, considering the kinetic aspect of transdermal absorption. Systemic absorption of topically applied nicotine decreased because of the dermal blood flow decreased by nicotine injected intravenously (Benowitz et al., 1992). Furthermore, the penetration of several compounds into deep dermis was promoted by the decrease in the cutaneous blood flow (Auclair et al., 1991). Taken all together, the attenuation of the skin blood flow could lead to the decrease in the systemic absorption and thus, lead to the increase in the local delivery of drugs after topical application. The ratio of antipyrine concentration in viable skin to the control tended to gradually increase from 1.8 to 2.7 with the increase in an applied dose of phenylephrine from 0.4 to 10 μmol, but the ratio was almost the same for the three compounds at 2 μmol phenylephrine (Fig. 3). On the other hand, the enhancement of muscular distribution was the largest for diclofenac (the ratio, 9.6), and salicylic acid (4.5) and antipyrine (3.3) followed (Fig. 3). This order is opposite to that for the absolute amount of drugs distributed to muscular layer (Fig. 2). The absorption of diclofenac to viable skin is the highest, but the subsequent penetration into muscular layer is the lowest, whereas the absorption to viable skin is the lowest and the muscular distribution is the highest for antipyrine (Fig. 2). This tendency has already been evidenced by $CL_{d\text{-}vs}$, a clearance from donor cell to viable skin, and $CL_{vs\text{-}m}$, a clearance from viable skin to muscular

layer, which were obtained by six-compartment analysis based on the results of the in vivo percutaneous absorption studies (Nakayama et al., 1999; Higaki et al., 2002). The largest enhancement was observed for diclofenac, because this compound is intrinsically removed from viable skin most effectively via the blood stream. To the contrary, the effect of the vasoconstriction was the smallest for antipyrine, because this drug is intrinsically most likely to penetrate into the muscular layer by avoiding being removed by the skin blood flow. The balance between removal by blood stream and direct penetration would determine the efficiency of the local penetration of drugs applied topically. In the previous study, we showed that drugs with larger unbound fraction in viable skin (fu_{vs}) are more likely to penetrate into muscular layer (Higaki et al., 2002). Actually, the value of fu_{vs} (27% homogenate) for antipyrine is the largest (0.998), whereas that for diclofenac is the lowest (0.592) among the three compounds (Higaki et al., 2002). These results, therefore, suggest that the approach utilizing the vasoconstriction could be more useful for drugs of which the muscular distribution is poorer. We suggested that increasing the unbound fraction could lead to the enhancement of local delivery of drugs applied topically in the previous study (Higaki et al., 2002), but we have shown that reducing the local blood flow in the skin would also be a promising approach to improve the penetration into muscular layer in the present study, although further studies would be needed to investigate if the vasoconstriction of the skin causes any serious adverse effect on the skin and the tissues around the application site.

References

Ahn, H., Johansson, K., Lundgren, O., Nilsson, G.E., 1987. In vivo evaluation of signal processors for laser Doppler tissue flowmeters. Med. Biol. Eng. Comput. 25, 207–211.

Auclair, F., Besnard, M., Dupont, C., Wepierre, J., 1991. Importance of blood flow to the local distribution of drugs after percutaneous absorption in the bipediculated dorsal flap of the hairless rat. Skin Pharmacol. 4, 1–8.

Barry, B.W., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 101–114.

Barry, B., 2002. Transdermal drug delivery. In: Aulton, M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. Harcourt Publishers Ltd., London, UK, pp. 499–533.

Benowitz, N.L., Jacob III, P., Olsson, P., Johansson, C.J., 1992. Intravenous nicotine retards transdermal absorption of nicotine: evidence of flood flow-limited percutaneous absorption. Clin. Pharmacol. Ther. 52, 223–230.

Catterall, W., Mackie, K., 1995. Local anesthetics. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Gilman, A.G. (Eds.), Goodman & Gilman's the Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 331–347.

Cross, S.E., Anderson, C., Thompson, M.J., Roberts, M.S., 1997. Is there tissue penetration after application of topical salicylate formulations? Lancet 350, 363.

Dawson, M., McGee, C.M., Vine, J.H., Nash, P., Watson, T.R., Brooks, P.M., 1988. The disposition of biphenylacetic acid following topical application. Eur. J. Clin. Pharmacol. 33, 639–642.

Higaki, K., Asai, M., Suyama, T., Nakayama, K., Ogawara, K., Kimura, T., 2002. Estimation of intradermal disposition kinetics of drugs. Part II. Factors determining penetration of drugs from viable skin to muscular layer. Int. J. Pharm. 239, 129–141.

Higaki, K., Amnuaikit, C., Kimura, T., 2003. Strategies for overcoming stratum corneum: chemical and physical approaches. Am. J. Drug Deliv. 1, 187–214.

Hoffman, B.B., Lefkowitz, R.J., 1995. Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Gilman, A.G. (Eds.), Goodman & Gilman's the Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 199–248.

Marty, J.P., Guy, R.H., Maibach, H.I., 1989. Percutaneous penetration as a method of delivery to muscle and other tissues. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption: Mechanisms, Methodology, Drug Delivery, second ed. Marcel Dekker, New York, pp. 511–529.

McNeill, S.C., Potts, R.O., Francoeur, M.L., 1992. Local enhanced topical delivery (LETD) of drugs: does it truly exist? Pharm. Res. 9, 1422–1427.

Millay, D.J., Larrabee Jr., W.F., Carpenter, R.L., 1991. Vasoconstrictors in facial plastic surgery. Arch. Otolaryngol. 117, 160–163.

M-Riviere, N.A., Inman, A.O., Riviere, J.E., McNeill, S.C., Francoeur, M.L., 1993. Topical penetration of piroxicam is dependent on the distribution of the local cutaneous vasculature. Pharm. Res. 10, 1326–1331.

Nakayama, K., Matsuura, H., Asai, M., Ogawara, K., Higaki, K., Kimura, T., 1999. Estimation of intradermal disposition kinetics of drugs. Part I. Analysis by compartment model with contralateral tissues. Pharm. Res. 16, 302–308.

Nilsson, G.E., 1984. Signal processor for laser Doppler tissue flowmeters. Med. Biol. Eng. Comput. 22, 343–348.

Radermacher, J., Jentsch, D., Scholl, M.A., Lustinetz, T., Frolich, J.C., 1991. Diclofenac concentrations in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease. Br. J. Clin. Pharmacol. 31, 401–418.

Singh, P., Roberts, M.S., 1993. Dermal and underlying tissue pharmacokinetics of salicylic acid after topical application. J. Pharmacokinet. Biopharm. 21, 337–373.

Urtti, A., Kyyronen, K., 1989. Ophthalmic epinephrine, phenylephrine, and pilocarpine affect the systemic absorption of ocularly applied timolol. J. Ocul. Pharmacol. 5, 127–132.