Regular Article

Effect of Iontophoresis on the Intradermal Migration Rate of Medium Molecular Weight Drugs

Kenji Sugibayashi,*,[a,b] Mika Futaki,[a] Miyu Hashimoto,[a] Asuka Fukuhara,[a] Kengo Matsumoto,[a] Takeshi Oshizaka,[b] Shoko Itakura,[a] and Hiroaki Todo[a]

[a] *Faculty of Pharmacy and Pharmaceutical Sciences, Josai University; Saitama 350–0295, Japan: and* [b] *Faculty of Pharmaceutical Sciences, Josai International University; Chiba 283–8555, Japan.*

The purpose of the present study was to evaluate whether iontophoresis (IP) accelerates the intradermal migration rate of medium molecular weight drugs. Sodium polystyrene sulfonate (PSA) and fluorescein isothiocyanate-dextran (FD) were used as model medium molecular weight acidic and non-electrolyte drugs, respectively. Low molecular weight acid and non-electrolyte drugs were also used for comparison. Drug-loaded excised split-layered skin (SL skin) was used in the experiment. SL skin was prepared using (i) whole skin was split once, (ii) the drug solution was applied on the lower skin, and (iii) the upper skin was layered onto the lower skin containing the drug solution as in the original skin. The effect of constant-current cathodal or anodal IP was applied to the SL skin, and the time course of the cumulative amount of drug migration from the SL skin through the dermis to the receiver was followed. In cases without IP and with anodal IP, the intradermal migration rates of medium molecular weight drugs were much lower than those of small molecules. The driving force for drug migration was thought to be simple diffusion through the skin layer. In contrast, cathodal IP significantly increased the intradermal migration rate of PSA not but of FD or low molecular weight drugs. This IP-facilitated migration of PSA was probably due to electrorepulsion. These results suggest that IP can be used to increase the intradermal migration of medium molecular weight charged drugs.

Key words iontophoresis; electrorepulsion; intradermal administration; intradermal migration; medium molecular charged drug

Introduction

Recently, many biopharmaceuticals comprised of medium to high molecular weight drugs have been produced. They include bioactive proteins and peptides produced by gene recombination and cell culture technologies, which have developed rapidly and become top selling pharmacological products around the world. In contrast, low molecular weight drugs of 300–500 Da are becoming less prominent.[1] However, most biopharmaceuticals are degraded or not absorbed in or from the gastrointestinal tract when administered orally.[2] In addition, biopharmaceuticals are generally expensive, which not only imposes an economic burden on patients but also has a negative impact on medical insurance systems in each country. Furthermore, patients require frequent visits to hospitals to receive drug treatment, because most biopharmaceuticals are administered by healthcare professionals. By requiring patients to visit and be hospitalized frequently, their potential working hours are reduced. A means to resolve these problems related to biopharmaceuticals may include: (i) development of biopharmaceuticals at a lower cost; (ii) sustaining the efficacy of biopharmaceuticals to avoid frequent administration; or (iii) development of self-administered biopharmaceuticals. Self-injectable formulations have already been developed for some biopharmaceuticals.[3] However, it is difficult to manage medication by self-injection, especially for infants and the elderly, who often need long-term care. In addition, infusion shock may occur when biopharmaceuticals are intradermally injected, so it is necessary to control the administration rate.

Another choice that can be self-administered easily, other than the oral formulations and self-injections, is use of transdermal drug delivery systems (TDDS). TDDS has merits such as maintaining the plasma level of a drug, avoiding first pass metabolism effects in the liver, and easy discontinuation of administration if side effects occur.[4] However, due to the stratum corneum barrier, the transdermal delivery rate of drugs is significantly lower than the absorption rate through the gastrointestinal mucosa. Against this background, solid microneedles (MN),[5] which can form micropores in the stratum corneum, and hollow MN,[6] which can infuse drug solutions by bypassing the stratum corneum into the cutaneous tissues have been developed recently using Bio-Micro Electro-Mechanical Systems. These tools have opened the possibility for the cutaneous tissues to administer biopharmaceuticals. Electroporation[7] and thermal poration[8] have also been tested as methods for forming micropores in the stratum corneum.

Even when such transdermal administration bypasses the stratum corneum barrier, medium to high molecular weight compounds must have a moderate to high intradermal migration rate (for easy accessibility to cutaneous microcapillaries). In other words, the migration rate of medium to high molecular weight compounds in the dermis layer becomes rate-determining for the overall systemic absorption.

We previously evaluated whether iontophoresis (IP) can increase the migration rate of medium molecular weight compounds in the dermis layer. IP has been broadly utilized to deliver drugs through the stratum corneum.[9–11] The promoting mechanism by IP is electrorepulsion and electroosmosis. In the present study, IP effects were determined and mathemati-

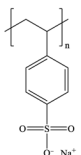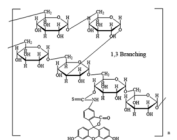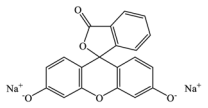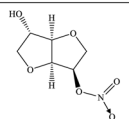* To whom correspondence should be addressed.   e-mail: sugib@josai.ac.jp

cally evaluated on the intradermal migration of medium molecular weight acidic and non-electrolyte (uncharged) drugs (about 4 and 10 kDa) using sodium polystyrene sulfonate (PSA) and fluorescein isothiocyanate-dextran (FD), respectively. Low molecular weight acid and non-electrolyte compounds were also used for comparison. Table 1 summarizes the model drugs used in this experiment. High molecular weight compounds were excluded in this study due to their extremely low intradermal migration rate.

Since MN were developed as a new tool to administer drugs by bypassing the stratum corneum as mentioned above, they have been used it to administer drugs into cutaneous tissues. Hollow type MN,[12] drug-coated solid MN,[13] and drug solubilized MN[14] have been designed for intradermal administration. The latter one dissolves immediately after injection. The length and thickness of the MN can be easily modified, so the puncture depth also varies.[12–14] In the present study, we set the injection depth of the drug solution to 400 μm.

Split-layered skin (SL skin) was prepared as follows: (i) whole skin (thickness: about 1000 μm) was split once at a thickness of about 400 μm from the skin surface, (ii) the drug solution was applied onto the lower skin (thickness: 600 μm), and (iii) the upper skin was layered back onto the lower skin containing the drug solution as in the original skin. The effect of constant-current cathodal or anodal IP was applied to the SL skin containing the drug, and the time course of the cumulative amount of drug migration from the SL skin through the dermis end to the receiver was followed over time.

Table 1. Chemical Structures and Molecular Weights of the Model Compounds Used in This Experiment

Drug category	Drug	Chemical structure	*M.W.*
Acidic Medium MW	PSA-4		4.3 k
	PSA-10		10 k
Non-electrolyte Medium MW	FD-4		3 – 5 k
	FD-10		10 k
Acidic Low MW	FL-Na		376.27
	ES		208.2
Non-electrolyte Low MW	ISMN		191.14

Experimental

Materials   Medium molecular weight acids, PSAs (M.W. 4.3 kDa, PSA-4 and M.W. 10 kDa PSA-10) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Medium molecular weight non-electrolytes, FDs (M.W. 3–5 kDa, FD-4 and M.W. 10 kDa, FD-10) were purchased from Sigma-Aldrich. In addition, low molecular weight acids, fluorescein sodium salt (FL-Na) and sodium 4-ethylbenzenesulfonate (ES) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), respectively. Low molecular weight non-electrolyte, isosorbide 5-mononitrate (ISMN) was purchased from Tokyo Chemical Industry Co., Ltd. Table 1 summarizes these structural formulas and molecular weights.

Hydroxypropyl cellulose (HPC, grade H) used as a formulation base was purchased from Nippon Soda Co., Ltd. (Tokyo, Japan). Other solvents and reagents were of special or HPLC grade without purification.

The ear skin from edible pigs was used in the experiment. The porcine ears were purchased from the Central Research Institute for Agricultural Feed and Livestock (Tsukuba, Ibaraki, Japan) and stored frozen at −30 °C until use.

Determination of Intradermal Migration Rate of Various Model Drugs

Applied Solution into Skin

PSA-4, PSA-10, FD-4, FD-10, ES, ISMN (10 mg/mL each), and FL-Na (0.5 mM) solutions were prepared using 5% HPC aqueous solution and used for intradermal application (see Table 1).

Preparation of SL Skin

Frozen porcine ear skin was thawed in warm water at 32 °C and carefully washed with purified water so as not to damage the skin surface. Excess moisture was wiped off and the skin was shaved with a clipper and shaver. Then, the porcine ear skin was cut off using a scalpel, and the fat on the dermis side was carefully removed using scissors, and the skin was sliced with a dermatome to adjust the thickness to 1000 μm from the skin surface. Then, the skin was sliced into two pieces, 400 μm upper skin and 600 μm lower skin. Drug solution (200 μL, see "Applied solution into skin") was applied onto the 600 μm lower skin (drug application area was 1.77 cm²), and the upper skin was layered onto the lower skin containing the drug solution as in the original skin to prepare split-layered (SL) skin. Skin thickness was determined using a thickness gauge (SM-112, Teclock Co., Ltd., Okaya, Nagano, Japan).

Determination of Drug Migration Rate

The SL skin was set in a vertical-type diffusion cell (receiver cell volume: 6.0 mL, effective diffusion area: 1.77 cm²). Phosphate buffered saline (PBS) (1/30 M, pH 7.4) (6 mL) was added to the receiver cell. During the drug migration test, the receiver cell was stirred constantly with a magnetic stirrer. Samples of the receiver solution (0.5 mL) were periodically collected, and the same amount of PBS was replenished at each sampling time to determine the time course of the cumulative amount of each drug migrating from the SL skin through the dermis end into the receiver cell. Since small molecular drugs diffuses faster than medium molecules through skin, the measurement period for small ones and medium ones were set to 4 to 6 h, 8 to 60 h, respectively. In addition, the fluorescein compounds FD-4 and FD-10 were not stable against the pH change, the measurement period for FD-4 and
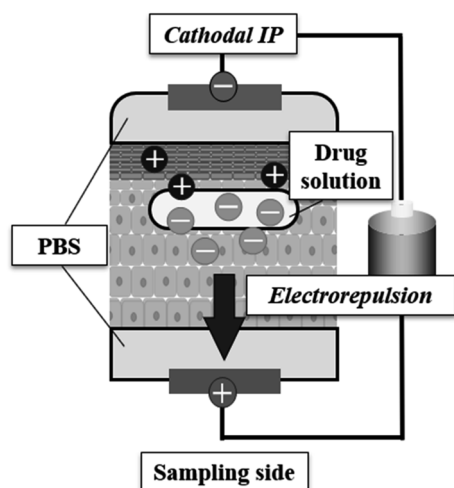
Fig. 1. Schematic Representation of the Experimental Setup for IP Application

Electrode setting in the case of cathodal IP (*vice versa* for anodal IP). Each electrode is in PBS, whereas the drug solution is applied in the skin tissue.

FD-10 were shorter than PSA-4 and PSA-10. The quantitative sensitivity of a fluorescent substance FD changes with the pH changes in the solution. Since the pH in the receiver cell changes when IP is used for a long period, the experiment was conducted in a short period with little pH change.

IP Application Figure 1 shows a schematic diagram of IP application. In the case of cathodal IP, the cathode was set on the stratum corneum and the anode was in the receiver cell. The electrodes were set the opposite way for anodal IP. A multichannel DC power supply VI1002 (Precise Gauge Co., Ltd., Hamamatsu, Japan) was used as an IP device. An Ag electrode and AgCl electrode were used for the anode and cathode, respectively. The voltage was set to deliver a current density of $0.3\,mA/cm^2$ (constant current IP). The voltage was applied throughout the drug migration experiments. The receiver solution was sampled periodically, and the drug concentration in the sample was measured using a fluorescence spectrophotometer, HPLC system, or ultra-HPLC (UHPLC).

Determination of Each Penetrant

FL-Na, FD-4 and FD-10

Receiver sample solution ($200\,\mu L$) containing FL-Na, FD-4, or FD-10 obtained in the diffusion experiment was transferred to a quartz glass cell (T-9-UV-10-1, Tosoh Quartz Corp., Yamagata, Japan) and assayed for fluorescence intensity using a fluorometer (RF-5300PC, Shimadzu Corp., Kyoto, Japan). The excitation and emission wavelengths for FL-Na were 485 and 535 nm, respectively, and those for FD-4 and FD-10 were 490 and 520 nm, respectively.

ISMN, PSA-4, and PSA-10

The receiver solution containing ISMN was mixed with

acetonitrile at a ratio of $1:1$ and that containing PSA-4 and PSA-10 was mixed with acetonitrile at a ratio of $6:4$, and then centrifuged at $21500\times g$ and $4\,°C$ for 5 min. Supernatant volumes of $10\,\mu L$ (for ISMN) or $30\,\mu L$ (for PSA-4 and PSA-10) were injected into the HPLC system, which consisted of a pump (LC-20AD, Shimadzu Corp., the same as below), an injector (SIL-20AC), a column oven (CTO-20AC), a UV-VIS detector (SPD-M20A) or a spectral fluorescence detector (RF-10AXL), a system controller (CBM-20AC), and an analytical data processing system (LC solution). All samples were quantified using the absolute calibration method. Table 2 summarizes the column, determination method, mobile phase, and its flow rate.

ES

The receiver solution containing ES was mixed with acetonitrile at a ratio of $3:1$ and centrifuged at $21500\times g$ and $4\,°C$ for 5 min. A sample of the supernatant ($8\,\mu L$) was injected into a UHPLC. The UHPLC system consisted of a pump (LC-30AD, Shimadzu Corp.), injector (SIL-30AC), column oven, UV-VIS detector, and a system controller (same type as above). The absolute calibration method was used. Other conditions are also shown in Table 2.

Analysis of Intradermal Migration Kinetics of Drugs The lower layer in the SL skin may be assumed to be a plane diffusion sheet, or the whole SL skin layer may be a drug matrix if drug diffusion immediately takes place. Then, the time course of the cumulative amount of drug migration from the SL skin through a unit area of the dermis end to the receiver, Q, was analyzed as a function of time, t, using a following general equation.[15]

$$Q = kt^n \qquad (1)$$

where k is the migration rate constant.

The n-value is 1.0 when the lower skin layer is assumed to be a plane diffusion sheet, but n is 0.5 when the SL skin becomes like the drug matrix and drug diffusion mainly contributes to drug migration.[16] The n-value becomes 1.0 when constant-current IP facilitates the intradermal migration of drugs, because drug migration is generally proportional to the electric current. In the present data analysis, $\log Q$ was plotted against $\log t$, excluding the data in the lag time period as follows:

$$\log Q = \log k + n \log t \qquad (2)$$

Results and Discussion

Effect of Electrode Setting for IP on the Intradermal Migration of Various Model Drugs Most of the following IP tests were performed in an *in vitro* system using excised SL skin, and the cathode (or anode) electrode was set on the stratum corneum surface and the anode (or cathode) was in the receiver solution, as shown in Fig. 1.[17]

Table 2. HPLC and UHPLC Conditions for Used Compounds in These Experiments

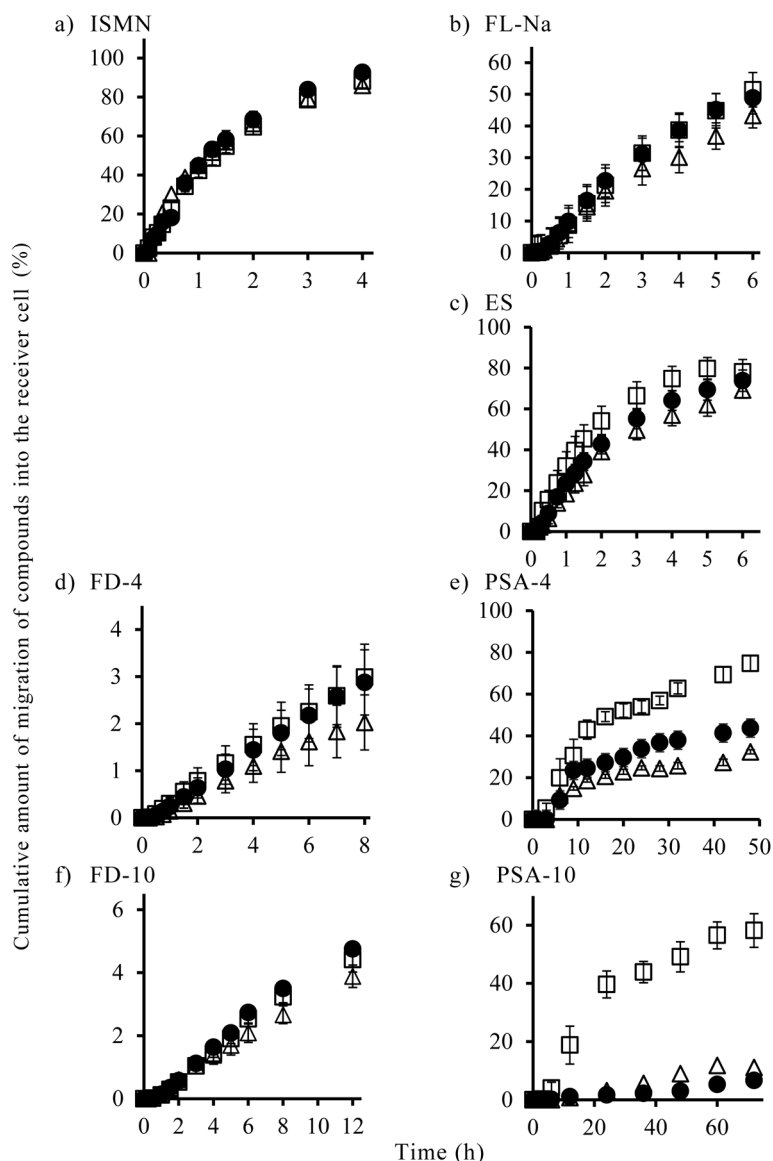|	ISMN/HPLC	PSA-4 or PSA-10/HPLC	ES/UHPLC
Column	Inertsil® ODS-3, $5\,\mu m$, $4.6\times150\,mm$ GL Sciences Inc., Tokyo, Japan	Asahipak GF-7M HQ, $9\,\mu m$, $7.5\times300\,mm$ Showa Denko K.K., Tokyo, Japan	Shim-pack GIS C18, $2\,\mu m$, $3.7\times75\,mm$ Shimadzu Corp., Kyoto, Japan
Detection	UV 220 nm	Ex 267 nm, Em 381 nm	UV 254 nm
Mobile phase	Acetonitrile : water $= 10:90$	Acetonitrile : 50 mM $Na_2PO_4\,aq. = 10:90$	Acetonitrile : 0.1% phosphoric acid $aq. = 25:75$
Flow rate	1.0 mL/min	0.6 mL/min	0.8 mL/min

Fig. 2. Effect of IP on the Time Course of the Cumulative Amount of Several Drugs Migrating from Excised Skin through the Dermis End to the Receiver

Symbols: □: cathodal IP, △: anodal IP, ●: without IP. Each point represents the mean ± standard error (S.E.) ($n = 4$).

Figure 2 shows the effect of constant-current IP on the cumulative amount of drugs migrated from the SL skin through the dermis end to the receiver cell. A high amount of small molecular non-electrolyte ISMN (Fig. 2a) and acidic FL-Na (Fig. 2b) and ES (Fig. 2c) was cleared from the SL skin without and with cathodal and anodal IP. The cleared percentage reached 70% or more of the injected dose of the drugs over 4–6 h. No electrorepulsion and electroosmosis effects were found with IP application. In other words, low molecular weight drugs, not only non-electrolyte (ISMN) but also acidic drugs (FL⁻, ES⁻), had high migration rates (high passive diffusion by concentration gradient) through the skin, so an IP effect was hardly found. In addition, the intradermal migration of medium molecular weight non-electrolytes FD-4 and FD-10 was not increased by cathodal and anodal IP (Figs. 2d, f). The cumulative amount of FD-4 and FD-10 migrated (a few percent against the applied dose) from skin was reduced to one-tenth compared with those for low molecular weight drugs (Figs. 2a–c). This was due to the limited electrorepulsion and electro-

osmosis for medium molecular weight FD-4 and FD-10.[18]

On the other hand, the intradermal migration of PSA-4 and PSA-10, which are medium molecular weight acidic drugs (Figs. 2e, g), was remarkably promoted by cathodal constant-current IP.[19] The cumulative amounts of PSA-4 and PSA-10 migrated by the cathodal IP increased to 70 and 60% of the dose, respectively, which were like the migration amount of low molecular weight compounds. Because anodal IP did not change the FD and PSA migrations from that without IP, the effect of electrorepulsion was very low. Compared with the cathodal IP efficacy (ratio with IP/without IP) on intradermal migration between PSA-4 and PSA-10, a higher efficacy was observed with PSA-10. These data were consistent with the report Guy *et al.*,[20] which showed that higher IP efficacy was observed with higher molecular weight compounds.

It is presumed that one of the reasons why PSA-4 showed higher migration than FD-4 is the presence or absence of charge of both molecules. That is, PSA-4 has a negative charge, while FD-4 is not ionized. Since the skin is negatively

charged, it is probable that the skin permeability of PSA-4 administered intradermally was higher than that of FD-4 due to electrical repulsion.[21,22]

Analysis of the Observed Intradermal Migration Rate of Drugs The observed intradermal migration rate of drugs was analyzed using n-values in Eq (2). Table 3 summarizes the calculated n-values. All cases tested with low molecular weight drugs showed $n \approx 0.5$ (0.43–0.69), not $n \approx 1$. These results suggested that low molecular weight drugs distributed almost evenly in the whole layer of SL skin (viable epidermis and dermis) except the stratum corneum immediately after drug application, and that the viable epidermis and dermis became like a drug-containing matrix to clear the drug from the SL skin through the dermis end into the receiver over time after the steady state period. On the other hand, medium molecular weight non-electrolytes (FD-4, FD-10) showed $n \approx 1.0$ (0.85–1.10) not $n \approx 0.5$. This result suggested that the dermis became a diffusion-controlled membrane for medium molecular weight drugs, resulting in membrane-controlled release.[15] In addition, n-value was also near 1.0 (0.69–1.04) when medium molecular weight acidic drugs (PSA-4, PSA-10) were applied to skin without IP or with anodal IP treatment. No difference was observed in the migration rates with and without anodal IP, suggesting that the migration rate was membrane-controlled. In addition, the amount of PSA-4 and PSA-10 cleared from the skin with cathodal IP was markedly higher than those with and without anodal IP. PSA flux from the dermis end during constant-current IP should be constant ($n = 1$) because the flux is generally proportional to the electric current as explained above. However, the n-value for PSA-4 and PSA-10 was near 1.0 (0.98 and 1.08) until 24 h, but then decreased with time to around 0.48 and 0.61, respectively. This was not due to a change in the intradermal migration mechanism but owing to the high consumption of the Ag and AgCl electrodes: *i.e.*, the Ag electrode became AgCl, whereas the AgCl electrode became Ag during the migration experiment. This high electrode consumption was determined by an increase in voltage at a constant-current IP (data not shown).

For a deeper understanding of the differences between the above-mentioned intradermal migration rates between low and medium molecular weight drugs, typical concentration–distance profiles of low and medium molecular weight molecules in the skin are illustrated in Figs. 3a and b, respectively. Detailed analysis will be shown in a separate paper because it goes beyond the scope of the present report. In addition, the main objective in the present study was to explain the effect of IP on the intradermal migration of medium molecular weight

drugs. Figure 3 shows schematic illustrations from post-lag time to about 8 h during the intradermal migration experiment shown in "Determination of drug migration rate." The migration coefficient of drugs in the 400 μm upper skin should be similar to that in the 600 μm lower skin,[23] but the barrier function of the stratum corneum is much higher than that in the viable epidermis and dermis. In other words, the migration rate in the stratum corneum was much lower than that in the viable epidermis and dermis. Thus, almost no drug was found in the stratum corneum and no concentration gradient of both low and medium molecular weight drugs was observed at the surface between the stratum corneum and viable epidermis ($dC/dx = 0$, see Figs. 3a and b). After a short lag time, both the low and medium molecular weight molecules distributed evenly in the 400 μm upper skin and a straight concentration gradient was obtained in the 600 μm lower skin to be zero at the interface to the receiver. The lag time for the low molecular weight molecules was shorter than that for the medium molecular weight molecules. The concentration–distance profile did not change much after the lag time to about 8 h for the medium molecular weight drugs, as shown in Fig. 3b. Therefore, almost zero-order migration rate with $n \approx 1.0$ was found for the medium molecular weight molecules (Fig. 2 and Table 3). On the other hand, the concentration–distance profile for the low molecular weight drugs changed with time, as shown in Fig. 3a. It was estimated that the migration profile of low molecular weight molecules from the dermis end to the receiver was similar to the drug release profile from the matrix as described above, with $n \approx 0.5$.

One of the goals is to have high bioavailability of intracutaneously applied drugs, so that we need to find out determinants for the high bioavailability of drugs. For orally administered drugs, the Biopharmaceutics Classification System (BCS) has become widely accepted as a bioequivalence source in the academic, industrial, and regulatory world.[24] The BCS classifies drugs into 4 types using 2 parameters (solubilities and gastrointestinal permeabilities). For transdermal application, the partition coefficient from the formulation to skin and diffusion coefficient through the stratum corneum of drugs were found to be the 2 biggest determinants for the percutaneous absorption rate. In contrast, no clear determinants were established for the intracutaneous absorption of drugs. Claassen reported that the absorption process from subcutaneous depots is determined by the diffusion of the compound into the extravascular tissue, by passage through the vessel wall, and by the capillary blood perfusion.[25] Because cutaneous tissues are richer in vessels than subcutaneous tissues, however,

Table 3. n-Value in Eqs. (1) and (2) for Several Drugs

Drug category	Drug	Drug conc.	n		
			Without IP	Cathodal IP	Anodal IP
Acidic medium MW	PSA-4	10 mg/mL	1.06	0.95	0.69
	PSA-10	10 mg/mL	1.01	1.08	1.04
Non-electrolyte medium MW	FD-4	10 mg/mL	1.08	1.01	0.85
	FD-10	10 mg/mL	1.10	1.02	0.94
Acidic low MW	FL-Na	0.5 mM	0.59	0.69	0.63
	ES	10 mg/mL	0.54	0.43	0.66
Non-electrolyte low MW	ISMN	10 mg/mL	0.57	0.65	0.56

Each n-value was obtained by double logarithmic plotting as follows: $\log Q = \log k + n \log t$ Eq. (2).

a) Small molecular weight drugs
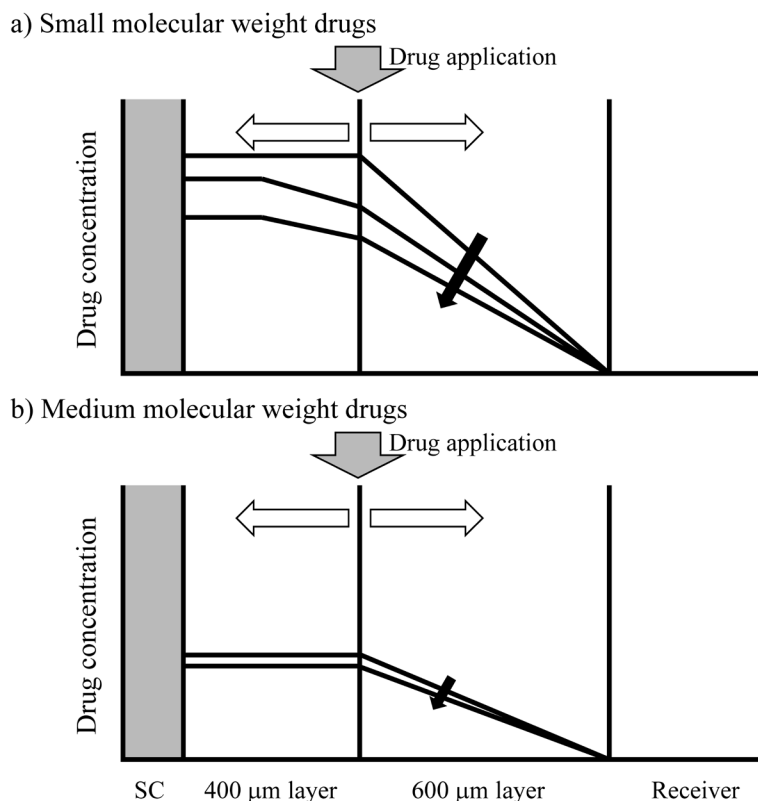

b) Medium molecular weight drugs

Fig. 3. Schematic Diagram of Concentration–Distance Profiles after Drug Application at $400\,\mu\mathrm{m}$ from the Skin Surface

Open arrows indicate the intradermal migration direction of drugs, and closed arrows indicate the concentration decrease with the passage of time after drug application.

capillary blood perfusion may not be a big determinant.[25]

On the intra- and sub-cutaneous absorption rate of drugs, Zuidema *et al.* reported the effect of drug lipophilicity,[26] and we published the dependency by molecular weight of drugs.[27] The migration rate of medium molecular weight drugs in cutaneous tissue was much lower than that of low molecular weight drugs, as shown in Fig. 2. Thus, IP, which enhances the intradermal migration rate of medium molecular weight electrolytes, may be an effective tool to increase their absorption rate and bioavailability.

IP is well known to increase the stratum corneum permeation of ionic drugs. The present study suggests that IP can be used also to increase the intradermal migration of medium molecular weight ionic drugs but not low molecular weight drugs.

Conclusion

It was clarified in the present study that the intradermal migration of medium molecular weight acidic drugs can be promoted by cathodal IP. This IP technology can be combined with recently developing devices such as MN[28] and needle-less syringes. Since such drug disposition and kinetics in the dermis change depending on the injection depth of the drug solution, it is important to further examine the effect of injection depth of drug solution on its disposition and kinetics. In addition, further consideration will be necessary to evaluate whether IP promotes vascular permeability and the lymphatic migration of middle molecular weight drugs.

Conflict of Interest The authors declare no conflict of interest.

References

1) Urquhart L., *Nat. Rev. Drug Discov.*, **19**, 228–228 (2020).
2) Liu C., Kou Y., Zhang X., Cheng H., Chen X., Mao S., *Expert Opin. Drug Deliv.*, **15**, 223–233 (2018).
3) Zhang Y., Yu J., Kahkoska A. R., Wang J., Buse J. B., Gu Z., *Adv. Drug Deliv. Rev.*, **139**, 51–70 (2019).
4) Pastore M. N., Kalia Y. N., Horstmann M., Roberts M. S., *Br. J. Pharmacol.*, **172**, 2179–2209 (2015).
5) McAllister D. V., Wang P. M., Davis S. P., Park J.-H., Canatella P. J., Allen M. G., Prausnitz M. R., *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 13755–13760 (2003).
6) Kim Y.-C., Park J.-H., Prausnitz M. R., *Adv. Drug Deliv. Rev.*, **64**, 1547–1568 (2012).
7) Prausnitz M. R., Bose V. G., Langer R., Weaver J. C., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 10504–10508 (1993).
8) Belsey N. A., Garrett N. L., Contreras-Rojas L. R., Pickup-Gerlaugh A. J., Price G. J., Moger J., Guy R. H., *J. Control. Release*, **174**, 37–42 (2014).
9) Batheja P., Thakur R., Michniak B., *Expert Opin. Drug Deliv.*, **3**, 127–138 (2006).
10) Bakshi P., Vora D., Hemmady K., Banga A. K., *Int. J. Pharm.*, **586**, 119584 (2020).
11) Wang Y., Zeng L., Song W., Liu J., *Drug Deliv. Transl. Res.*, (2021), in press.
12) Wang P. M., Cornwell M., Hill J., Prausnitz M. R., *J. Invest. Dermatol.*, **126**, 1080–1087 (2006).
13) Gill H. S., Prausnitz M. R., *J. Control. Release*, **117**, 227–237 (2007).
14) Lee J. W., Park J. H., Prausnitz M. R., *Biomaterials*, **29**, 2113–2124 (2008).
15) Ritger P. L., Peppas N. A., *J. Control. Release*, **5**, 23–36 (1987).
16) Higuchi W. I., *J. Pharm. Sci.*, **51**, 802–804 (1962).
17) Numajiri S., Sugibayashi K., Morimoto Y., *Chem. Pharm. Bull.*, **44**,

1351–1356 (1996).

18) Wu X.-M., Todo H., Sugibayashi K., *J. Control. Release*, **118**, 189–195 (2007).

19) Marro D., Guy R. H., Delgado-Charro M. B., *J. Control. Release*, **70**, 213–217 (2001).

20) Guy R. H., Kalia Y. N., Delgado-Charro M. B., Merino V., López A., Marro D., *J. Control. Release*, **64**, 129–132 (2000).

21) Morimoto Y., Kokubo T., Sugibayashi K., *J. Control. Release*, **18**, 113–122 (1992).

22) Hosoya O., Sano M., Wada Y., Seki T., Sugibayashi K., Juni K., Morimoto Y., *Chem. Pharm. Bull.*, **46**, 882–885 (1998).

23) Sugibayashi K., Todo H., Oshizaka T., Owada Y., *Pharm. Res.*, **27**, 134–142 (2010).

24) Dahan A., Miller J. M., Amidon G. L., *AAPS J.*, **11**, 740–746 (2009).

25) Claassen V., *Tech. Behavioral Neural Sci.*, **12**, 35–45 (1994).

26) Zuidema J., Kadir F., Titulaer H. A. C., Oussoren C., *Int. J. Pharm.*, **105**, 189–207 (1994).

27) Yoshida D., Todo H., Hasegawa T., Sugibayashi K., *Eur. J. Pharm. Sci.*, **35**, 5–11 (2008).

28) Lee H., Song C., Baik S., Kim D., Hyeon T., Kim D. H., *Adv. Drug Deliv. Rev.*, **127**, 35–45 (2018).