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Transdermal administration of salmon calcitonin by pulse depolarization-iontophoresis in rats

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Abstract

Using the pulse depolarization-iontophoresis (PDP-IP) system, salmon calcitonin (sCT), a drug for the treatment of osteoporosis, was transdermaly administered in rats. While absorption of sCT was not observed after passive transdermal administration, the serum sCT concentration was confirmed at adose of 0.2–4 μg when the PDP-IP system was employed. The results indicated that PDP-IP could enhance transdermal absorption of peptide drugs. Also noted was the increased amount of absorption of sCT along with an increase in the dose. We investigated the influence of electrical parameters (current, frequency) in PDP-IP on the transdermal absorption of sCT. An optimal current for drug absorption was found within the range of transported current (0.1–1.0 mA) employed for PDP-IP. In comparison with the results obtained at 0.1 mA, the drug absorption increased, along with an increase in transported current, when the current was set at 0.5 mA, while the drug absorption decreased at 1.0 mA in comparison. The decrease in drug absorption was assumed to be attributable to the structural destruction of skin by application of excessive current. There was no change in skin resistance attributable to the frequency; nor was there any influence of the frequency on the amount of drug absorption.

Keywords: Bioavailability; Pulse Depolarization; Iontophoresis delivery; Salmon calcitonin; Transdermal administration

1. Introduction

Calcitonin consists of 32 amino acids. It is a peptide hormone which inhibits bone resorption by inhibiting the activity of osteoclasts. Similar to activated vitamin D3, estrogen and bisphosphonate, calcitonin is used as a drug for the treatment of osteoporosis. When orally administered, calcitonin is easily degraded by the enzymes in the gastrointestinal tract. Due to its high hydrophilic property and high molecular weight, the absorption of calcitonin from the intestinal mucosa is poor (Lee and Yamamoto, 1990). Calcitonin is

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clinically administered by intramuscular injection, even though side effects such as nausea and facial flushes are caused by a high blood concentration peak after injection. Furthermore, intramuscular injection has problems such as infection at the time of injection, pain caused by needle insertion and poor patient compliance.

To avoid the above problems, transdermal administration or transmucosal administration were proposed to take the place of intramuscular injection (Lee et al., 1994). Iontophoresis is a method of positively feeding ionized drugs into the skin or mucosa by providing an electrical field to the skin or mucosa. This method enables transdermal and transmucosal administration of large molecular weight drugs. Accordingly, the method is now attracting attention as a non-invasive administration method for peptide drugs. Drug delivery under direct current type or pulse type electrical conditions have been investigated in many studies on iontophoresis. Reports have been made on the transdermal administration of high molecular weight drugs such as insulin, vasopressin and LHRH (Green, 1996; Heit et al., 1994).

The pulse depolarization iontophoresis (PDP-IP) system is a unique system which provides a method of depolarizing the skin surface after application of each pulse. This PDP-IP system is expected to decrease skin irritation by depolarization of the skin surface and efficient delivery of drugs. Using the PDP-IP system, metoprolol (M.W. 267) which is a low molecular weight β-blocker was transdermally delivered in human subjects (Okabe et al., 1986).

In this study, we transdermally administered salmon calcitonin (M.W. 3431.9), which is a high molecular weight drug, using the PDP-IP system in rats to investigate the potency of the PDP-IP system as a non-invasive drug delivery system. We compared the amount of drug absorption during and after PDP-IP administration with that observed after intramuscular administration, which is the clinically used method at present. We also investigated the influence of each electrical parameter on transdermal drug absorption when the PDP-IP system was employed and discussed the change of electrical properties in skin caused by the PDP-IP system.

2. Materials and methods

2.1. Materials

Salmon calcitonin (sCT) (0.2 μg/IU) was purchased from Nova Biochem, Inc. (Philadelphia, PA). Hisamitsu ADIS4030, the electric source device for PDP-IP, was purchased from Advance Co., Inc. (Tokyo, Japan). SD male rats (7–8 weeks old, 211–326 g/body) were purchased from Oriental Bio-Service (Tukuba, Japan). For the determination of serum sCT concentration by radioimmunoassay (RIA), a calcitonin RIA kit (RIA6003) was purchased from Penninsula Laboratories, Inc. (Belmont, CA). For the determination of the serum calcium concentration by the *o*-cresolphthalein complexone method, Calcium-C Test Wako was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

2.2. Applicator

The anode applicator [(+)-applicator] comprised three layers of silver electrode, conduction layer and drug retaining membrane and the applicable area was 2.5 cm^2. A cation exchange membrane (A-201) purchased from Asahi Chem. Ind. Co., Ltd. (Japan) was used in the conduction layer for the purpose of preventing the transition of the silver ion to the skin. The cathode applicator [(-)-applicator] comprised silver/silver chloride electrode and conductive gel and the applicable area was 2.5 cm^2. Polyvinyl alcohol gel, 12% (w/w), containing 0.9% (w/w) NaCl was used as the conductive gel. As the isoelectric point (pI) of sCT is 9.7 and sCT is charged positive in the neutral field, the drug retaining membrane on the anode side was impregnated with 10 μl of sCT in distilled water containing each dose (0.2, 0.4, 1, 2 and 4 μg).

2.3. PDP-IP system

Constant direct current type iontophoresis (CDC-IP) is conventionally used. The PDP-IP system is different from CDC-IP, because it employs a pulse type galvanization system and is equipped with a function to remove the current

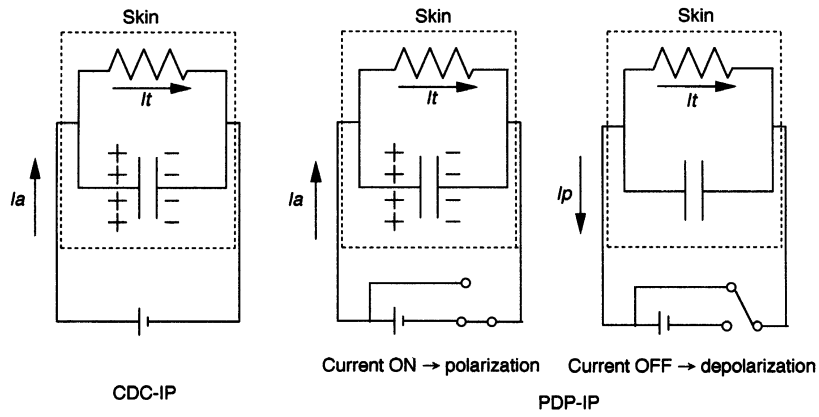

Fig. 1. Electric circuit of CDC-IP and PDP-IP. I_a denotes applied current, I_t denotes transported current and I_p denotes polarized current.

accumulated on the skin surface after the application of each pulse.

In general, the skin condition is expressed by the parallel circuit of the resistance and condenser (Yamamoto and Yamamoto, 1976). When iontophoresis is applied to the skin, the current is divided into one which is accumulated in the condenser part and another which transports the resistance part. By short circuiting the current after application of each pulse, PDP-IP removes the current accumulated in the condenser part of the skin at the time of current application (Fig. 1). In Fig. 1, the current applied to the skin (applied current, I_a), that actually transported the skin (transported current, I_t) and that removed from the skin (polarized current, I_p) are expressed.

2.4. Electrical conditions

PDP-IP was performed using Hisamitsu ADIS-4030. Fig. 2 shows the typical current waveforms when PDP-IP was applied. First, the current expressed as 'Area A' was applied to the skin for current applying time ($t2$-$t1$). Then the 'Area B' current was passed in the opposite direction for depolarizing time ($t3$-$t2$) by short circuiting. These two procedures were repeated at a adequate frequency. The current obtained by subtracting 'Area B' from 'Area A' per pulse ($t3$-$t1$) was the current actually transported through the skin. A decrease in current caused by polarization was observed immediately after application of voltage. However, because of the discharge of accumulated electric charge by depolarization, high current might be applied in the next pulse.

$$I_a = \text{Area A}/(t3\text{-}t1) \quad (1)$$

$$I_t = (\text{Area A} - \text{Area B})/(t3\text{-}t1) \quad (2)$$

in which $t1$ denotes the application time (duration) of a certain pulse current and $t3$ the next pulse current application time. I_a denotes applied current (mA) and I_t transported current (mA). 'Area A' and 'Area B' denote the amount of

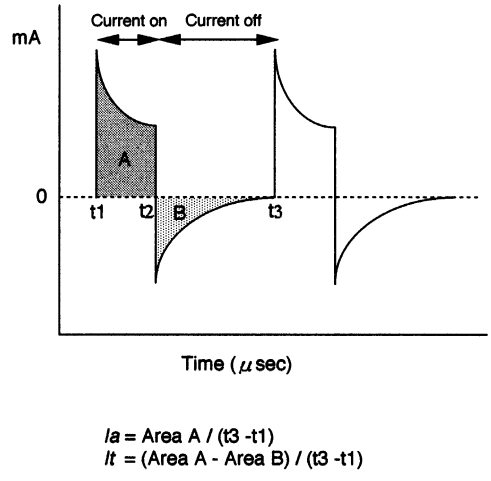

Fig. 2. Typical current wave pattern of PDP-IP, showing that the skin is depolarized after each pulse.

current (mA·s), respectively. The time ratio of current application (duty cycle, %) was calculated as $(t2\text{-}t1) \times 100/(t3\text{-}t1)$. All the experiments were conducted by applying constant voltages or currents. In an experiment to investigate the influence of frequency, the frequency was set at 1, 10 and 50 kHz, but in the remaining experiments 30 kHz was chosen. The duty cycle was set at 30% in all the experiments. The current applying time was 45 min. In a constant voltage experiment, the current values (I_t and I_a) were determined every 5 min and the mean value was calculated. As electrical features of skin, the resistance (R) and impedance (Z) were calculated from the following equations. In this study, the resistance (R) was defined as the resistance against the current which passed through only the resistance of the skin (Eq. (3)), and impedance (Z) was defined as the resistance against the current which passed though the resistance and accumulated in the condenser of the skin (Eq. (4)).

$$\text{Resistance } (R) = (v/I_t) \times \text{duty}/100 \quad (3)$$

$$\text{Impedance } (Z) = (v/I_a) \times \text{duty}/100 \quad (4)$$

in which v denotes voltage (V) and duty denotes the voltage-applied time ratio (%) during a pulse cycle.

2.5. Animal experiments

2.5.1. Transdermal administration experiment by PDP-IP

Iontophoretic transdermal administration was conducted in SD male rats using PDP-IP. The application site was the abdomen, which was shaved by an electric razor or hair clippers the day before application. Before the application of drug, the animals were macroscopically confirmed to have no damage in the application site. The animals whose stratum corneum was damaged were prepared by the tape stripping method (Tsai et al., 1991) in which adhesive tape (CELLOPHAN Tape™, Nichiban, Japan) was attached and peeled 20 times. The animals were anesthetized by intraperitoneal injection of aqueous ethyl carbamate solution (25% w/w, 5 ml/kg), after which the (+)-applicator and (−)-applicator were applied to the abdomen. Then a prescribed voltage was applied for a prescribed time, after which both applicators were detached. Time course blood collection from the jugular vein was performed before and during voltage application, as well as after detachment of both applicators. The blood was immediately placed in a polypropylene low adsorptive test tube with a siliconized surface and was left standing over ice for about 60 min. The blood was then centrifuged (15 000 rpm, 5 min) to collect serum which was stored at −20°C. The serum sCT concentration was determined by RIA. The serum calcium concentration was determined using a calcium measurement kit to check the pharmacological activity of sCT after administration by PDP-IP.

2.5.2. Intravenous administration (iv) experiment

sCT was dissolved in distilled water with 0.01% (w/v) BSA. Eighty-five–95 μl of a 2.5 or 5.0 μg/ml sCT solution was administered into the caudal vein of the anesthetized rats. Time course blood collection from the jugular vein was then performed. After blood collection, the serum sCT concentration was determined by the same procedure, as employed after transdermal administration by PDP-IP.

2.5.3. Intramuscular administration (im) experiment

sCT was dissolved in 0.9% (w/v) saline with 0.01% (w/v) BSA. Ninety-five–105 μl of 2.5 or 5.0 μg/ml sCT solution was administered into the femoral muscle of the anesthetized rats. Time course blood collection from the jugular vein was then performed. After blood collection, the serum sCT concentration was determined by the same procedure, as employed after transdermal administration by PDP-IP.

2.6. Data analysis

Using the measured blood concentration data, the AUC values obtained after intravenous (iv) and intramuscular (im) administrations, and transdermal administration by PDP-IP, were calculated by the trapezoidal method. The AUC obtained after transdermal administration by

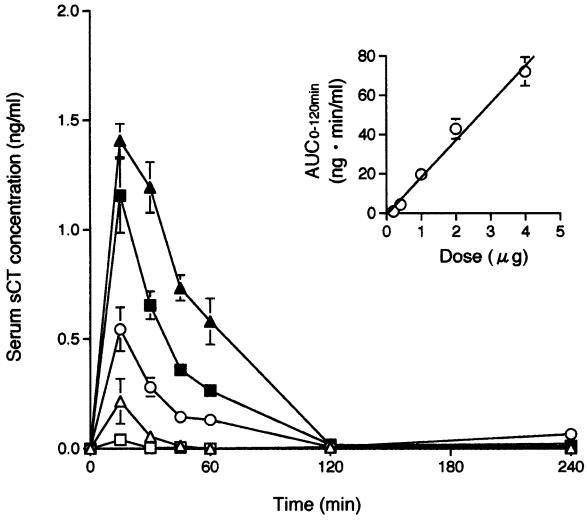

Fig. 3. Serum sCT concentration during and after PDP-IP of sCT administration of 0.2(■), 0.4(○), 1(●), 2(△), and 4(▲) μg in rats. Constant voltage (12 V) was applied for 45 min by PDP-IP (30 kHz, duty 30%) (mean ± SE, $n = 4$). Inset: Relationship between dose and AUC of sCT.

PDP-IP was compared with the iv and im values, and absolute and relative bioavailabilities (BA) were calculated, respectively. In this calculation, the BA of iv was expressed as absolute BA ($AUC_{IP}/AUC_{iv} \times Dose_{iv}/Dose_{IP} \times 100$) and the BA of im as relative BA ($AUC_{IP}/AUC_{im} \times Dose_{im}/Dose_{IP} \times 100$).

AUC_{IP}, AUC_{iv} and AUC_{im} represent the AUCs of serum sCT concentration after administration by PDP-IP, iv and im, respectively. $Dose_{IP}$, $Dose_{iv}$ and $Dose_{im}$ represent the doses administered by PDP-IP, iv and im, respectively.

3. Results

3.1. Transdermal absorption of sCT by PDP-IP

Fig. 3 shows the serum sCT concentrations in rat at each dose (0.2–4 μg/body), when PDP-IP was applied for 45 min at a constant voltage of 12 V. A rapid increase in blood concentration was observed at each dose and C_{max} was observed during application of PDP-IP. Also confirmed was the linearity between the dose up to 4 μg/body and $AUC_{0-120\ min}$. On the other hand, no absorption of sCT was observed in the transdermal administration experiment at 2 μg/body without using PDP-IP (passive penetration) (data is not shown). Table 1 shows the BA at each dose. The BA against iv (that is absolute BA) was 32.5–37.0%. The BA against im (which is the clinically employed method at present) (that is relative BA) was 67.2–76.5%.

The transported current (I_t) during PDP-IP at a constant voltage of 12 V for 45 min was about 0.3–0.7 mA. Under these conditions, no skin irritation was macroscopically observed in the site where PDP-IP was applied. From the standpoint of drug absorption and skin irritation, the usefulness of PDP-IP, as a peptide delivery system, was suggested.

After the application of PDP-IP, the amount of drug which remained in the applicator detached from the skin was determined. The results indicated that about 12% of the initial content remained in all the cases (i. e., about 88% of the drug in the applicator was released). The results of absolute BA and the released amount of drug from the applicator did not correspond with each other.

Table 1
Comparison of AUC among several administration routes of sCT in rats (mean ± SE)

Administration route	Dose (μg/kg)	$AUC_{0-120\ min}$ (ng·min/ml)	Absolute BA (%)	Relative BA (%)	n
iv	2	34.2 ± 1.9	(100)	–	3
im	2	16.6 ± 2.2	48.4	(100)	4
PDP-IP	3.5[a]	19.8 ± 2.9	33.2	68.6	4
PDP-IP	6.8[a]	43.0 ± 5.0	37.0	76.5	4
PDP-IP	13.0[a]	72.2 ± 7.3	32.5	67.2	4

[a] 1, 2 and 4 μg of SCT was administered per rat.

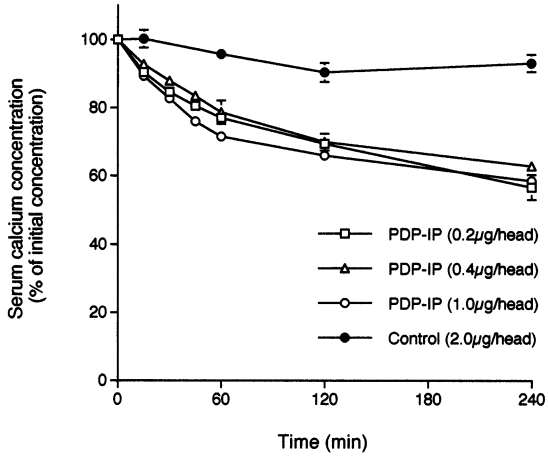

Fig. 4. Reduction of serum calcium concentration during and after PDP-IP administration of sCT of 0.2(□), 0.4(△), and 1(○) μg in rats. Constant voltage (12 V) was applied for 45 min by PDP-IP (30 kHz, duty 30%) except control. Control experiment was conducted without PDP-IP [2.0 μg sCT(●)] (mean ± SE, $n = 4$).

3.2. Pharmacological activity of sCT

For the purpose of investigating the pharmacological activity of sCT after administration by PDP-IP, the serum calcium concentration was determined. Fig. 4 shows the serum calcium level at each dose. The decrease in serum calcium concentration was immediately observed after the start of PDP-IP and the decrease of about 30–40% against the initial value was noted at 240 min after application, confirming the drug efficacy of sCT administration by PDP-IP. There was no significant difference in the decrease of serum calcium concentration among different dose studies.

3.3. Influence of transported current (I_t) on drug absorption

Fig. 5 shows the relationship between I_t (0.1, 0.5 and 1 mA) applied and the AUC obtained afterwards. The transport of the ionized drug by iontophoresis was assumed to be dependent on the current which passes the resistant part of skin. However, the AUC of sCT decreased when I_t exceeded 0.5 mA in this experiment. While the AUC was 51 ng·min/ml when I_t was 0.5 mA, the AUC was 40 ng·min/ml when I_t was 1.0 mA. Concerning the site of application, a macroscopically detectable thermal burn was noted in the skin after application at 1.0 mA for 45 min. Histological investigation indicated atrophy of epidermal cells and uniform amorphism of dermal collagen fibers. In order to investigate the influence of skin condition on drug absorption, the transdermal administration experiment was performed by applying 0.2 mA constant I_t and 8 V constant voltage to the tape-stripping skin in rats. Even though a higher mean I_t (1.9 mA) was obtained at 8 V constant voltage in the tape-stripping skin rat, the AUC was much lower than the AUC obtained after the application of 1.0 mA to intact skin rat. Fig. 6 shows the impedance (Z) and resistance (R) of the skin at various currents. Along with the increase in I_t, the skin resistance decreased, suggesting a decrease in the dermatological barrier function. On the other hand, no substantial change in the impedance was observed at each I_t. The skin impedance is a parameter formed by resistance and capacitance (electricity accumulated in the condenser) of the skin. Due to the structural change in the skin by application of a high current, the resistance decreased (this

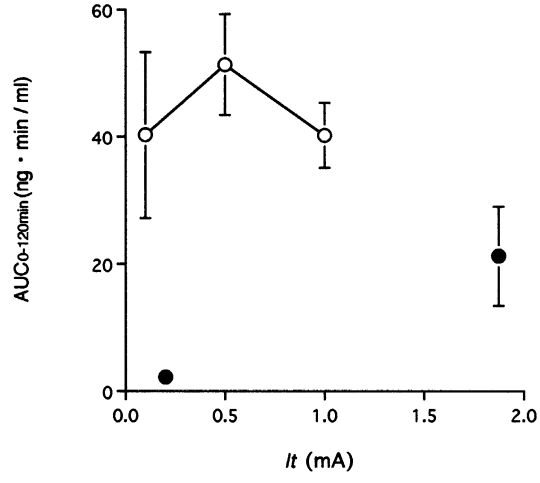

Fig. 5. Relationship between I_t and AUC in intact skin rat (○) and stripped skin rat (●). Two μg of sCT was administrated by PDP-IP (30 kHz, duty 30%) for 45 min. PDP-IP was applied at constant current (I_t: 0.1, 0.2, 0.5, and 1.0 mA) and at constant voltage (8V, mean I_t: 1.9 mA) (mean ± SE, $n = 4$).

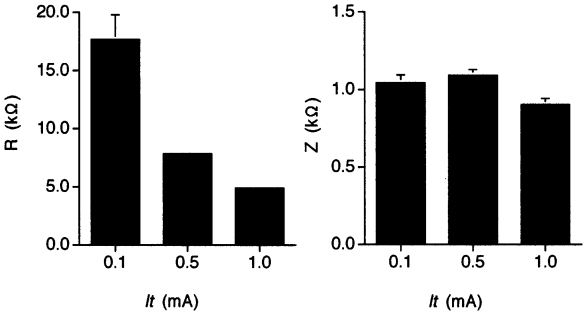

Fig. 6. Resistance (R) and impedance (Z) during PDP-IP administration of sCT at constant current in rats (I_t: 0.2, 0.5, and 1.0 mA). PDP-IP (30 kHz, duty 30%) was applied for 45 min (mean ± SE, $n = 4$).

makes impedance decrease). However, this structural change also caused a decrease in the capacitance (this makes impedance increase), so that the difference between the impedance at a low current and that at a high current was apparently small.

3.4. Influence of frequency on drug absorption

Fig. 7 shows the influence of frequency on drug absorption. No significant difference was observed between the AUCs obtained at three frequencies. Fig. 8 shows the results of electric features in the skin at each frequency. While the impedance went down along with an increase in frequency, there was no significant difference in the resistance at each frequency. In other words, the frequency of PDP-IP did not influence the skin resistance involved in the drug transport.

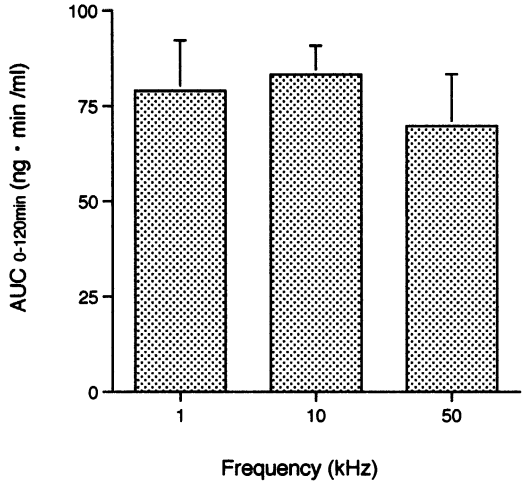

Fig. 7. Effect of frequency on AUC of sCT in rats. PDP-IP (8V, duty 30%) was applied for 45 min at constant voltage (mean ± SE, $n = 4$).

4. Discussion

In this study, we conducted transdermal administration of sCT in rat using PDP-IP, for the purpose of investigating the usefulness of the PDP-IP system. We also investigated the influence of various factors including the dose of drug, transported current and frequency on the drug absorption. The transdermally absorbed amount of sCT after administration at each dose (0.2–4 μg/body), using PDP-IP, increased along with the dose. Theoretically, the drug absorption by iontophoresis is known to be described by the following equation (Sage, 1995).

$$J_{sct} = t_{sct} \times (I_t/Z_{sct}/F)$$
$$= (Z_{sct}\cdot\mu_{sct}\cdot C_{sct}/(Z_{sct}\cdot\mu_{sct}\cdot C_{sct} + A)) \times (I_t/Z_{sct}/F) \quad (5)$$

In this formula, J_{sct} is the transport rate of ionized sCT by the current I_t, t_{sct} is the transport number of sCT, Z_{sct} is the ion value of sCT, F is the Faraday constant, μ_{sct} is the mobility of sCT, C_{sct} is the mol concentration of sCT in the preparation, A is the total sum ($\Sigma_{zc\mu}$) of the product of mobility, mol concentration and ion value of all ions involved in the electrical skin transmission other than sCT.

This equation indicates the involvement of the transport number on the influence of drug concentration in the preparation on the drug absorption. The transport number is proportional to the drug concentration in the preparation within a certain concentration range ($Z_{sct}\cdot\mu_{sct}\cdot C_{sct} << A$). The increase in this transport number was assumed to be partly responsible for the increase in drug absorption along with an increase in the drug concentration in this study. In addition to the transport number, another reason could be the convective solvent flow, which occurred at the time of iontophoresis application. When ions are electrically passed through a charged membrane,

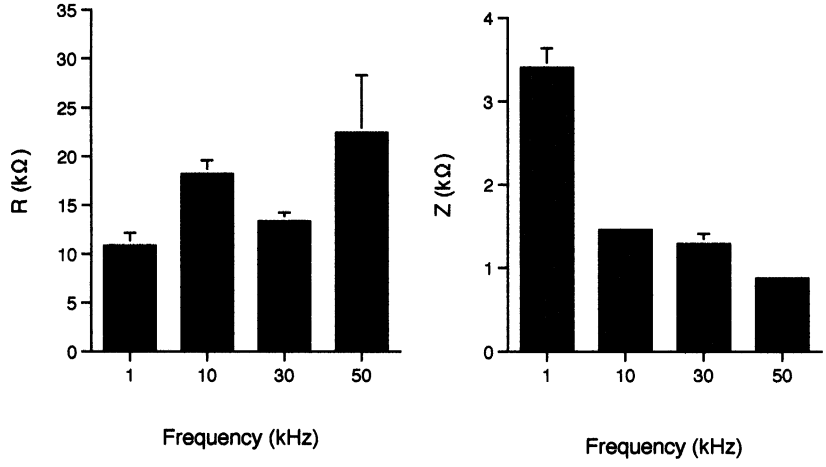

Fig. 8. Relationship between frequency and resistance (R), impedance (Z) during PDP-IP administration of sCT in rats. PDP-IP (8V, duty 30%) was applied for 45 min at constant voltage (mean $\pm$ SE, $n = 4$).

a solvent flow called electro-osmosis is caused (Aveyard and Haydon, 1973). The drug transport by current and that by convective solvent flow were involved in the drug absorption at the time of iontophoresis application (Mathot et al., 1989), and the latter is considered to be dependent on the drug concentration. The overall drug transport seemed to increase through convective solvent flow caused by an increase in drug concentration.

A decrease in serum drug concentration (T_{max}; 15 min) was observed at each dose during application of PDP-IP. The residual drug in the applicator detached from the skin after application was 21.9% after 15 min, 12.8% after 30 min and 12.2% after 45 min. Based on the change in the rate of the residual drug with the passage of time, the decrease in serum drug concentration was assumed to be attributable to the depletion of drug in the applicator. The BA of sCT did not correspond to the absorbed amount (about 88%) calculated from the residue, probably because of the first pass effect, including the metabolism in the skin and the binding.

In general, for the purpose of checking the pharmacological activity of sCT (hypocalcemic effect) after transdermal administration, the decreased rate of serum calcium concentration in rats was used as an index. In many reports, the decreased rate by the administration of sCT in rats was about 15 to 25% and the calcium concentration returned to the initial value several hours after drug administration. For example, when 7 μg/kg of human calcitonin was administered intravenously to hairless rats in an experiment conducted by Thysman et al., the serum calcium concentration decreased by about 15% at 120 min after administration, but returned to the initial level at 360 min after administration (Thysman et al., 1994). In this study, the level decreased by about 30–40% against the initial value at 240 min after application. The main reason the serum calcium concentration decreased more after administration of a smaller dose of sCT by PDP-IP, than after iv administration of hCT, may be the difference in drugs. Both sCT and hCT comprise 32 amino acids, but they are structurally differ from each other at several amino acid positions. The clinically effective dose of sCT is about 50 times less than that of hCT (Chang et al., 2000). In our experiment, no significant difference was observed in serum calcium concentration when different doses (0.2, 0.4, 1 and 2 μg/head) were administered. The results suggested that the drug efficacy during and after application of PDP-IP demonstrated the maximum effect at each dose.

Concerning the influence of electrical factors on the transdermal absorption of drug, it is clear from Eq. (5) that the transdermal absorption of the drug is proportionally dependent on the current which passes through the skin. In fact, in the case of administration of sCT by iontophoresis using the epidermis separated from human cadaver skin, a linear relationship with a high correlation coeffcient between flux and current density (0.1–0.75 mA/cm^2) was observed (Chang et al., 2000). However, our results indicated the opposite result, that is, the drug absorption decreased at a higher current. Since a similar tendency was observed in animals whose stratum corneum was stripped, the decrease of the drug absorption was assumed to be attributable to the structural change of the stratum corneum in the skin by application of a high current. In fact, the resistance, macroscopic findings and histological observation of the skin to which a high current (I_t: 1.0 mA) was applied, indicated structural change in comparison with the skin to which a low current (I_t: 0.1, 0.5 mA) was applied. Also, in administration of lidocaine hydrocholoride by iontophoresis, the drug permeability decreased in the damaged skin whose stratum corneum was stripped compared with that of the skin with intact stratum corneum (Riviere, 1986). In general, the skin is a negatively charged membrane and can selectively allow the transport of positively charged ions (Sage, 1995). The stratum corneum of nude mice is permselective and the permselectivity is roughly 1.6: 1 for cations (Burnette and Ongpipattanakul, 1987). This selectivity also causes convective solvent flow, that assists the diffusion of positively charged ions and impedes that of negatively charged ions. The poor drug absorption at a high current seemed to be attributable to the disappearance of the ion selectivity along with the change in the membrane structure of the skin.

Since there is skin irritation, skin damage or uncomfortable electrical stimulation when iontophoresis is applied to man, a maximum current of 0.5 mA/cm^2 is commonly used (Banga and Chien, 1988; Burnette and Ongpipattanakul, 1988; Ledger, 1992). In our experiment, definite skin damage was observed after application of a 0.4 mA/cm^2 (1.0 mA/2.5 cm^2) current. The reason for this may be that the stratum corneum of the rats was thinner and structurally more susceptible to current, than that of man. In human skin also, excessive current may induce a phenomenon similar to that observed in rats.

Concerning the frequency, which is the other electrical parameter, it is reported that the transport of drug through the membrane is dependent on the frequency in the general pulse type current and that maximum drug transport is observed at a frequency of about 4 kHz, and that the electrical transport of drug in the membrane is decreased at a higher frequency (Nakhare et al., 1994). However, the influence of frequency on sCT was not observed in the PDP-IP system employed in our experiment. Nor was any influence on the resistance of skin noted. There was a decrease in impedance, but this decrease was assumed to be attributable to an increase in the capacitance (electricity accumulated in the condenser) of skin along with the increase of frequency. It is still not clear how the influence of frequency on skin properties is changed by the presence or absence of the depolarization function. It is necessary to investigate the change in skin resistance associated with a change in frequency of the non-depolarization pulse type current.

PDP-IP promoted sufficiently the absorption of sCT, which is not absorbed by passive transdermal delivery, indicating the usefulness of PDP-IP. The results suggest the possibility of the development of a noninvasive peptide delivery system. As to the influence of each electrical factor of PDP-IP on the transdermal absorption of sCT, an optimal current was confirmed, which is considered to serve as useful information in the future clinical application of this system.

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