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RESEARCH PAPER

Iontophoresis-Facilitated Delivery of Prednisolone through Throat Skin to the Trachea After Topical Application of its Succinate Salt

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ABSTRACT

Purpose The possibility of direct delivery of steroids through the skin to the trachea and the effect of iontophoresis on delivery efficacy were evaluated after the application of an ionic steroidal prodrug, prednisolone sodium succinate (PS-Na), to the throat skin.

Methods Fluorescein sodium salt (FL-Na) and PS-Na were applied as model compounds at a concentration of 1% in pH 7.4 phosphate-buffered saline to the throat skin of hairless rats, and constant current-cathodal iontophoresis (0.4 mA/cm^2) was performed for 8 or 10 h.

Results *In vitro* permeation experiment involving cathodal iontophoresis through excised hairless rat abdominal skin revealed 30- and 10-times higher levels of skin permeation of PS and its active drug, prednisolone (P), than those induced without iontophoresis. *In vivo* iontophoresis treatment of the rat's throat skin produced 2.6-, 1.6- and 12-times higher FL, PS and P concentrations, respectively, in the trachea than those observed without iontophoresis.

Conclusion The present results suggest the usefulness of topical application of the ionic steroidal prodrugs onto throat skin followed by iontophoresis treatment for directly delivering the steroid to the trachea.

KEY WORDS direct delivery · iontophoresis · skin permeation · steroidal prodrug · trachea

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INTRODUCTION

The pathology of bronchial asthma involves chronic inflammation and related constriction/increased sensitivity of the respiratory tract. In addition, the increase in chronic inflammation results in remodeling, such as smooth-muscle hypertrophy, hyperplasia in the glandular mucosa, vascular proliferation, and basement membrane thickening (1,2). Although bronchial asthma and cough-variant asthma were thought to be due to bronchial inflammation, inflammation in the trachea also had to be taken into account (3,4). Inhaled steroids are the first-choice treatment for achieving long-term control of asthma and preventing asthma-induced remodeling (1,5,6). However, the side effects associated with inhaled steroids, such as hoarseness and stomatitis, make this therapy difficult (7). In addition, the steroidal concentration in the trachea is lower than in the bronchi, resulting in the lower therapeutic effects on the trachea.

The aim of the present study is to evaluate the efficacy of the direct delivery of steroids through skin and the effects of iontophoresis (IP) on direct delivery. Several studies have already been performed on the direct delivery of drugs through skin to the subcutis and underlying muscle (8–10). We have also studied the possibility of direct delivery and its delivery ratio of topically applied drugs into the muscle through the skin (11,12). Singh and Roberts found that the subcutaneous tissue concentration of topically applied steroids under the application site was much higher than that at the tissues at the opposite site and the plasma concentration (13).

The use of vasoactive agents and IP treatment was found to increase the drug concentrations of deeper tissues after the topical application of drugs (14–16). IP is an electrical-based skin-penetration-enhancing method. Electrorepulsion and electroosmosis are the two major mechanisms by which IP enhances skin penetration of drugs

(17,18). Electrorepulsion is the primary mechanism used to increase the skin permeation of ionic compounds, and electroosmosis enhances solvent flow through cation-selective membranes, such as the skin (19). Murray *et al.* reported the direct delivery of topically applied water-soluble glucocorticoids to subcutaneous lesions via IP application (20). As the cervical (throat) skin is very close to the trachea, the direct delivery of topically applied drugs from the skin surface to the trachea might be possible when systemic absorption from the cutaneous and subcutaneous tissues into vessels can be avoided or is negligible.

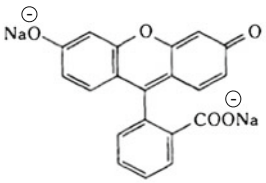
In the present study, the possibility of the targeted delivery of topically applied anti-inflammatory drugs to the trachea was evaluated using IP technology in order to obtain better therapeutic effects during asthma care. First, the aqueous fluorescent dye fluorescein sodium salt (FL-Na) was selected as a model compound and applied to the throat skin *in vivo* with cathodal IP treatment in order to evaluate the possibility of direct delivery of the compound (fluorescent intensity) to the trachea in hairless rats. Since FL in the trachea might migrate not only from the skin surface but also via the systemic circulation, the abdominal application of FL-Na was also studied with cathodal IP treatment. Next, a steroidal anti-inflammatory prodrug, prednisolone sodium succinate (PS-Na), was used to evaluate the direct delivery of PS to the trachea through the skin using cathodal IP treatment. PS was previously found to be metabolized to the active steroid prednisolone (P) by esterase (21,22). *In vitro* skin permeation experiments using PS and P were then performed with and without cathodal IP treatment. *In vivo* experiments were also carried out to evaluate the delivery rate and amounts of PS and P delivered through the skin to the trachea. Skin barrier function in atopic dermatitis patients is lower than those in healthy people. Some of the asthma patients have low skin barrier function like atopic dermatitis patients compared with patients with healthy full-thickness skin. Thus, we observed the effect of barrier strength on the enhanced delivery of PS and P with IP through the throat skin using stratum corneum-stripped skin as well as full-thickness skin. Finally, the ability of IP treatment to target steroidal drugs to the trachea was evaluated and discussed.

MATERIALS AND METHODS

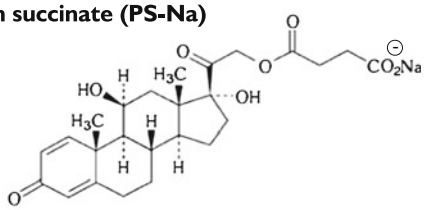
Materials

Fluorescein sodium salt (FL-Na), prednisolone sodium succinate (PS-Na), and prednisolone (P) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.) (Fig. 1). All other chemicals and solvents were of reagent grade or HPLC grade and used without further purification.

Fluorescein sodium salt (FL-Na)
MW 376.30
logP -0.615
pKa 6.4



Prednisolone sodium succinate (PS-Na)
MW 482.5
ClogP -1.01
pKa 0.3-0.95



Prednisolone (P)
MW 360.45
logP 1.62

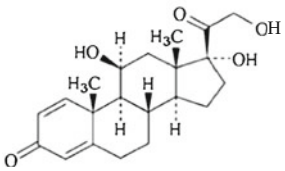


Fig. 1 Chemical structures of fluorescein sodium salt, prednisolone sodium succinate, and prednisolone.

Experimental Animals

Male hairless rats (WBM/ILA-Ht, weight: 220–280 g) were obtained from the Life Science Research Center of Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animals (Fukaya, Saitama, Japan). All animal feeding procedures and experiments were approved by the Institutional Animal Care and Use Committee of Josai University.

In Vivo Application of Prednisolone Sodium Succinate or Fluorescein Sodium Salt to the Throat or Abdominal Skin in Hairless Rats

A polypropylene tube (with a height of 2.0 cm and an effective diffusional area of 0.64 cm^2) (Asahi Glass Co. Ltd., Tokyo, Japan) was applied to the throat of anesthetized (by *i.p.* injection of 1.0 g/kg urethane) hairless rats (hair had been shaved before the experiment) using cyanoacrylate adhesive (Fig. 2a). In addition, one more tube was applied to the chest skin (Fig. 2a). Separately, two tubes were applied to the right and left abdomen (Fig. 2b). Stratum corneum-stripped skin and full-thickness skin were used. The stripped skin was made by stripping the stratum corneum off 20 times with adhesive tape. One percent FL-Na or 1% PS-Na in pH 7.4 phosphate-buffered saline (PBS) (700 μL) was added to the polypropylene tube applied to the throat skin or one of the tubes applied to the abdominal skin (cathodal side), whereas the same volume of pH 7.4

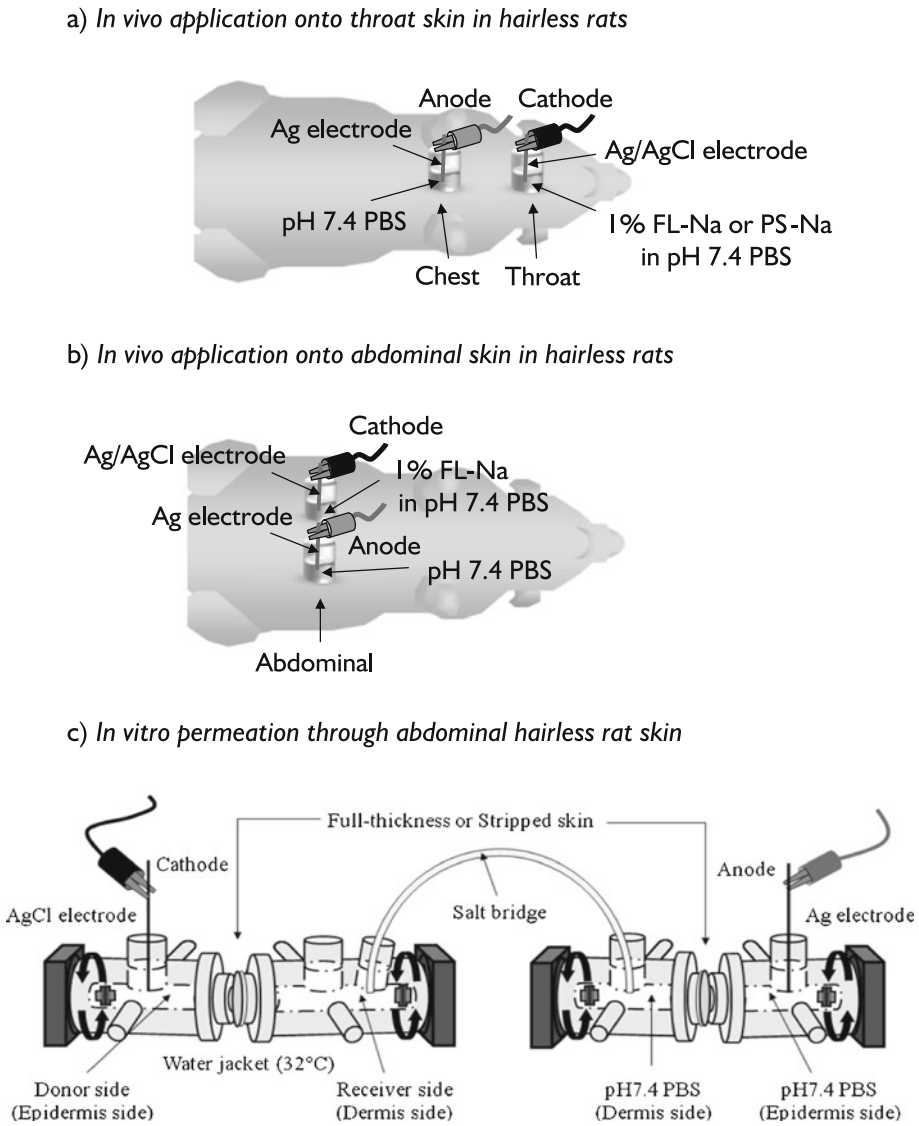


Fig. 2 Experimental setup for the *in vivo* hairless rat application experiment (**a** and **b**) and *in vitro* permeation experiment through hairless rat skin (**c**).

PBS was applied to the chest skin or the other tube applied to the abdominal skin (anodal side). In the case of cathodal IP treatment, an AgCl electrode (cathode electrode) was applied to the drug donor, and an Ag electrode (anode electrode) was applied to the PBS solution. Since the electric current must be less than 0.5 mA/cm^2 for safety (23), the current was adjusted to 0.4 mA/cm^2 and applied for 8 h. An ADIS-HP (Hisamitsu Pharmaceutical Co., Inc., Tokyo, Japan) was used to supply the electric. Blood was withdrawn from the jugular vein 8 h after skin application of the drugs. Then, the animal was killed, and the remaining blood was removed. The throat skin, sternohyoid muscle, and trachea were excised in this order. Plasma was obtained after centrifugation of the blood at 18800×g for 5 min at 4°C. The obtained plasma and tissues were kept in a freezer at −20°C. The frozen tissues were then cut into pieces using scissors and homogenized with water under ice. To the resulting 1% homogenate (200 μL), the same volume of internal standard solution (1.0 μg/mL triamcinolone acetonide in acetonitrile) was added for P and PS determination (or acetonitrile alone for FL determination), thoroughly mixed and centrifuged to obtain the supernatant. PS and P concentrations were then determined using an LC/MS (API300 or LCQ DECA XP plus), and FL was determined at excitation and emission wavelengths of 485 and 535 nm, respectively, using a fluorescent spectrophotometer (RF5300PC, Shimadzu, Kyoto, Japan).

Fluorescence Observation of the Trachea Surface after Throat Skin Application *In Vivo*

The trachea was excised from the hairless rats where FL-Na was topically applied and fixed on a glass plate (trachea surface upside). The fluorescence of the trachea sample was observed under fluorescent microscopy (IX71,

Olympus, Tokyo, Japan), and its intensity was analyzed using ImageJ (National Institute of Mental Health, Bethesda, MD, U.S.A.).

In Vitro Abdominal Skin Permeation of Prednisolone Succinate

Hairless rats were anesthetized by *i.p.* injection of 50 mg/kg sodium pentobarbital, and their abdominal skin (full-thickness or stripped skin) was excised. Excess fat was removed using scissors and hydrated in pH 7.4 PBS for an hour. Then, the skin sample was placed in a side-by-side diffusion cell (effective diffusion area: 0.95 cm^2). One percent PS-Na/pH 7.4 PBS (3.0 mL) was added to the epidermis side, and the same volume of PBS alone was added to the dermis side to start the skin permeation experiment (24). In the case of cathodal IP treatment, two side-by-side diffusion cells, each containing a skin sample, were used, as illustrated in Fig. 2c. Similarly, 3.0 mL of 1% PS-Na/pH 7.4 PBS and PBS alone were added to the epidermis (cathode) and dermis (anode) side, respectively. AgCl and Ag electrodes were used as the cathode and anode, respectively, for the IP. Both dermis sides were connected by a salt bridge (1% agar in 3.3 N KCl solution). Constant current (0.4 mA/cm^2) cathodal IP was applied during the skin permeation experiment (10- and 8 h-experiments were performed for the full-thickness skin and stripped skin permeation, respectively). The diffusion cells were maintained at 32°C by warm water circulation around the cells. The solution in each diffusion cell was stirred using a stirrer bar and a magnetic stirrer throughout the experiments. Aliquots (0.5 mL) were periodically sampled from the dermis side (cathode and dermis side for the IP treatment), and the same volume of PBS was added to ensure the volume constant.

The same volume of internal standard solution (10 μg/mL propyl *p*-hydroxybenzoate in acetonitrile) was added to the receiver sample and centrifuged to obtain the supernatant. The PS and P concentrations in the supernatant were determined by HPLC. To measure the skin concentrations of PS and P, each skin sample was taken and frozen at −20°C after the skin permeation experiments. The frozen skin was cut using scissors and homogenized with pH 7.4 PBS under ice. The PS and P concentrations in the obtained supernatant were also determined by HPLC.

Determination of Prednisolone Succinate and Prednisolone

The HPLC system used to determine the PS and P concentrations in the receiver solution consisted of a pump (LC-20AS; Shimadzu), a column (Capcell pak C18, UG120 5 μm, 4.6×250 mm; Shiseido, Tokyo, Japan), an auto-injector (SIL-20A; Shimadzu), a UV detector (SPD-20A; Shimadzu), and an analysis system (LC solution; Shimadzu). The mobile phase was composed of 0.032% tetra-*n*-butyl ammonium bromide, 0.32% $NaH_2PO_4 \cdot 12H_2O$, and 0.69% KH_2PO_4 : methanol (21:29), and the flow rate was 1.0 mL/min. The column was kept at room temperature.

The PS and P concentrations in the tissues were determined by LC/MS. The LC/MS system used for the measurement of PS consisted of a pump (LC-10 AD; Shimadzu), a column (Develosil ODS UG-3, Nomura Chemical, Aichi, Japan), and an API300 (Takara Shuzo Co., Ltd., Kyoto, Japan). The mobile phase was composed of 1 mM ammonium acetate : acetonitrile (65:35), the flow rate was 1.0 mL/min, and the column was kept at room temperature. The LC/MS system used for the measurement of P consisted of a pump (Paradigm MS4; AMR Inc., Meguro, Tokyo, Japan), an auto-injector (HTS-PAL; CTC Analytics AG, Zwingen, Switzerland), and an LCQ DECA XP plus (Thermo Fisher Scientific K.K., Yokohama, Kanagawa, Japan). The column was a Hypersil GOLD 3 μm, 2.1× 50 mm (Thermo Fisher Scientific K.K.), the mobile phase was composed of an A : B mixed solution (65:35) (A: acetonitrile : distilled water : formic acid=90:10:0.1, B: acetonitrile : distilled water : formic acid=2:98:0.1), the flow rate was 0.2 mL/min, and the column was kept at room temperature.

Statistical Analysis

Each data point is shown as the mean ± S.E. of three to eight experimental runs. Statistical analysis was performed by analysis of variance (ANOVA) with non-repeated measures or Mann-Whitney U-test, and $P<0.05$ was assumed to be significant.

RESULTS

Direct Delivery of Fluorescein to the Trachea *In Vivo*

The ability of our method to directly deliver drugs to the trachea through throat skin was evaluated using a model anionic compound, FL. One percent FL-Na/pH 7.4 PBS was applied to the throat skin of hairless rats and administered via cathodal IP for 8 h. Constant current (0.4 mA/cm^2) IP was used. Figure 3 shows the FL concentrations obtained in each tissue. The FL concentrations in the throat skin, sternohyoid muscle, trachea, and plasma in the IP group (8366, 36.4, 136, and 24.5 nmol/g, respectively) were about 1.5, 1.5, 2.6, and 65 times higher, respectively, than those in non-IP group (6164, 23.5, 51.5, and 0.38 nmol/g, respectively). Figure 4 shows fluorescent photos of the trachea surface in the IP group and non-IP group. Higher fluorescence was observed at the trachea

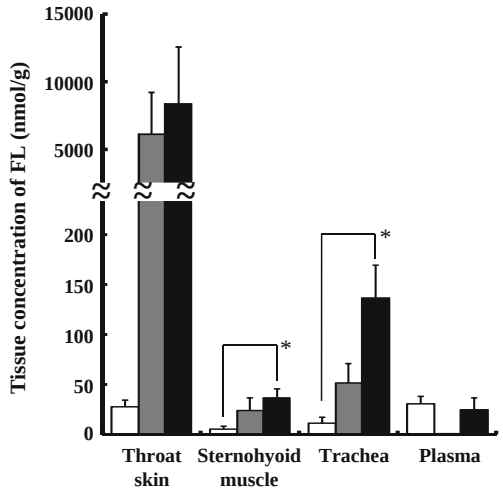


Fig. 3 Amount of fluorescein retained in each tissue sample excised from hairless rats 8 h after the application of 1% fluorescein sodium salt/pH 7.4 PBS to full-thickness throat or abdominal skin with or without cathodal iontophoresis. Each column represents the mean ± S.E. ($n = 3–5$). Statistical analysis was performed by ANOVA with nonrepeated measures. *$P < 0.05$ was assumed to be significant. Symbols: □: abdominal skin for IP group, ■: throat skin for non-IP group, ■: throat skin for IP group.

surface in the IP group than in the non-IP group. The fluorescent intensity (17.0±2.7) of the tissue from the IP group was higher than that (11.7±1.9) of the tissue from the non-IP group. These results suggested that FL delivery through the skin to the trachea was enhanced by cathodal IP treatment. However, a high plasma concentration of FL was also observed after IP treatment. Thus, the redistribution of the FL from the systemic circulation to the trachea may not be negligible. We then evaluated the redistribution of FL that had been absorbed through the skin and passed into the systemic circulation after the topical application of 1% FL-Na/pH 7.4 PBS to the abdomens of hairless rats and subsequent IP treatment for 8 h. Figure 3 also shows these results. The plasma concentration of FL 8 h after its application to the abdominal skin (30.0 nmol/mL) with IP was almost the same as that after its application to the throat skin with IP. On the other hand, the FL concentrations of the throat skin, sternohyoid muscle, and the trachea (27.5, 4.89, and 11.2 nmol/g, respectively) 8 h after IP application to the abdominal skin were 0.003, 0.14, and 0.08 times, respectively, of those seen after their IP application to the throat skin. Thus, the FL in the trachea mainly came from IP-facilitated direct delivery through the throat skin, although redistribution from the systemic circulation may also have been slightly involved.

In Vitro Skin Permeation of Prednisolone Succinate

Next, similar experiments were carried out using the acidic prednisolone prodrug PS-Na. Since PS is metabolized to P by esterase in the skin, the effects of cathodal IP on the *in vitro* permeation of PS as well as its metabolite, P, through full-thickness or stripped abdominal skin were evaluated. The cumulative amounts of PS, P, and their total (PS+P) that permeated through full-thickness skin over 10 h in the IP group were 131, 117, and 248 nmol/cm^2, respectively, and these values were 30, 10, and 15 times higher than those observed in the non-IP group (4.2, 12.2, and 16.4 nmol/cm^2) (Fig. 5 and Table 1). In addition, the skin concentrations of PS, P, and their total in the IP group (245, 501, and 746 nmol/g, respectively) were about 4, 9, and 6 times higher, respectively, than those seen in the non-IP group (62, 59, and 121 nmol/g, respectively) (Table 2). Stratum corneum-stripped skin was used as a model of the skin with low barrier function. The cumulative amount of PS that permeated through the stripped skin over 8 h in the IP group (1434 nmol/cm^2) was similar to the corresponding value in the non-IP group (1281 nmol/cm^2). However, that of P and the cumulative total of PS and P in the IP group (1183 and 2617 nmol/cm^2, respectively) were 2.8 and 1.5 times higher, respectively, than those in the non-IP group (428 and 1709 nmol/cm^2, respectively) (Fig. 6 and Table 1). The skin concentrations of PS, P, and their total in the IP group (7642, 1548, and 9191 nmol/g, respectively) were

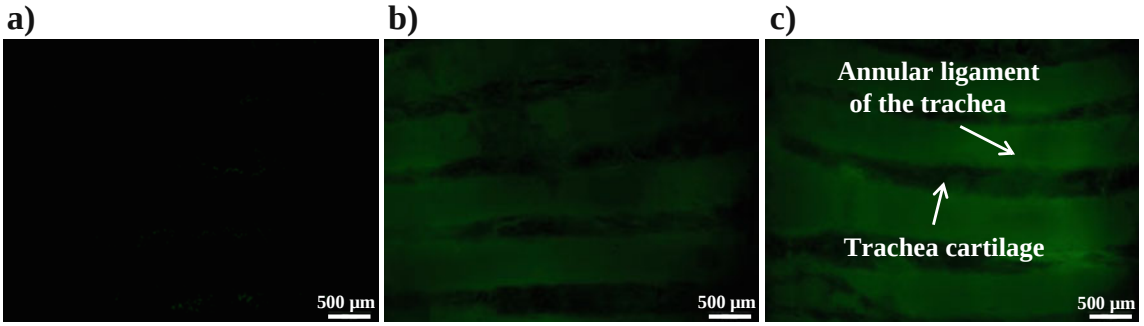


Fig. 4 Fluorescent observation of the outside surface of the trachea after the *in vivo* application of 1% sodium fluorescein/pH 7.4 PBS to the throat skin of hairless rats. **a** Non-application, **b** non-IP group, and **c** IP group.

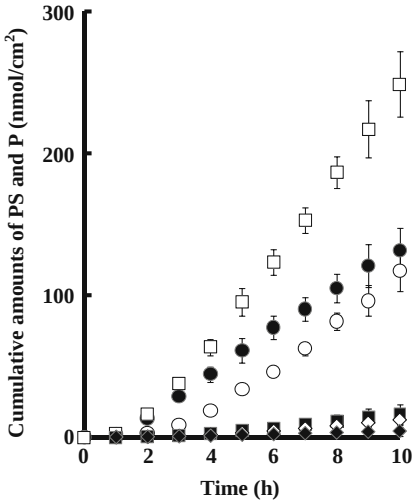


Fig. 5 Effects of cathodal iontophoresis on the time course of the cumulative amounts of prednisolone succinate and prednisolone that permeated through full-thickness skin from 1% prednisolone sodium succinate solution. Each point represents the mean ± S.E. ($n = 3$–6). Symbols: non-IP group; ■: PS+P, ♦: PS, ◊: P, IP group; □: PS+P, ●: PS, ○: P.

similar to those in the non-IP group (8010, 1576, and 9586 nmol/g, respectively) (Table 2).

In Vivo Application of Prednisolone Sodium Succinate to the Throat Skin in Hairless Rats

The present IP treatment increased the skin permeation and skin concentration of P as well as PS. Next, 1% PS-Na/pH 7.4 PBS was applied to the throat skin of hairless rats in order to evaluate the possibility of direct delivery of PS and P into the trachea through throat skin and the ability of cathodal IP to increase the efficacy of PS and P delivery in *in vivo* situations. Figures 7 and 8 show the results obtained after PS-Na application to full-thickness throat skin and stripped skin, respectively. Although the PS concentrations in the sternohyoid muscle and plasma after its application to the full-thickness throat skin in the IP group (3.2 nmol/g and 0.19 nmol/mL, respectively) were similar to those in the non-IP group (5.4 nmol/g and 0.21 nmol/mL, respectively), the PS concentrations in the throat skin and trachea in the IP group (228 and 6.7 nmol/g, respectively) were 2 and 1.6 times higher, respectively, than those in the non-IP group (119 and 4.1 nmol/g, respectively) (Fig. 7a). Interestingly, the P concentrations in the throat skin, sternohyoid muscle, and trachea in the IP group (401, 6.9, and 8.5 nmol/g, respectively) were markedly higher (13, 23, and 12 times, respectively) than those in the non-IP group (31.4, 0.3, and 0.7 nmol/g, respectively). In addition, the plasma concentration of P in the IP group (0.03 nmol/mL) was much lower than that in the non-IP group (0.11 nmol/mL) (Fig. 7b). However, no big effect of the IP application of PS onto stripped throat skin on the PS concentrations in the throat skin, sternohyoid muscle, trachea, or plasma was observed (1080, 10.5, 11.1, and 1.09 nmol/mL for IP group,

Table 1 Cumulative Amount of Prednisolone Succinate and Prednisolone that Permeated through Skin 10 h or 8 h after Their Application to Full-Thickness Skin or Stripped Abdominal Skin

	Full-thickness skin		Stripped skin	
	non-IP group	IP group	non-IP group	IP group
PS (nmol/g)	4.0 ± 3.2	125 ± 15[a]	1217 ± 347	1362 ± 189
P (nmol/g)	11.6 ± 3.5	112 ± 14[a]	407 ± 104	1124 ± 510
PS+P (nmol/g)	15.6 ± 6.6	237 ± 22[a]	1624 ± 300	2486 ± 540

Each point represents the mean ± S.E. ($n = 3$–6)

[a] Significant at $P < 0.05$ when compared with non-IP. Statistical analysis was performed by Mann-Whitney U-test.

Table 2 Skin Concentrations of Prednisolone Succinate and Prednisolone 10 h or 8 h after Their Application to Full-Thickness Abdominal Skin or Stripped Skin

	Full-thickness skin		Stripped skin	
	non-IP group	IP group	non-IP group	IP group
PS (nmol/g)	62.4 ± 8.4	245 ± 40[a]	8010 ± 1412	7642 ± 586
P (nmol/g)	59.3 ± 15.3	501 ± 67[a]	1576 ± 88	1548 ± 124
PS+P (nmol/g)	121 ± 22	747 ± 48[a]	9587 ± 1457	9191 ± 567

Each point represents the mean ± S.E. ($n = 3$–8)

[a] Significant at $P < 0.05$ when compared with non-IP. Statistical analysis was performed by Mann-Whitney U-test

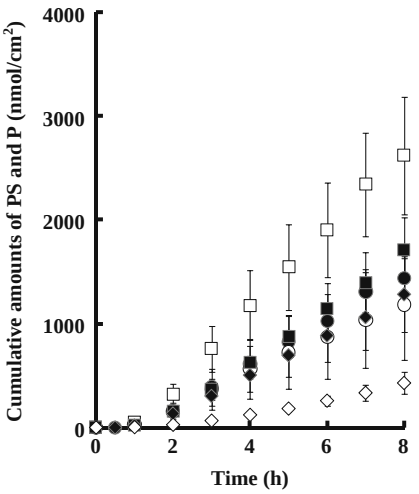


Fig. 6 Effects of cathodal iontophoresis on the time course of the cumulative amounts of prednisolone succinate and prednisolone that permeated from 1% prednisolone sodium succinate solution through stripped skin. Each point represents the mean ± S.E. ($n = 3$–6). Symbols: non-IP group; ■: PS+P, ♦: PS, ◊: P, IP group; □: PS+P, ●: PS, ○: P.

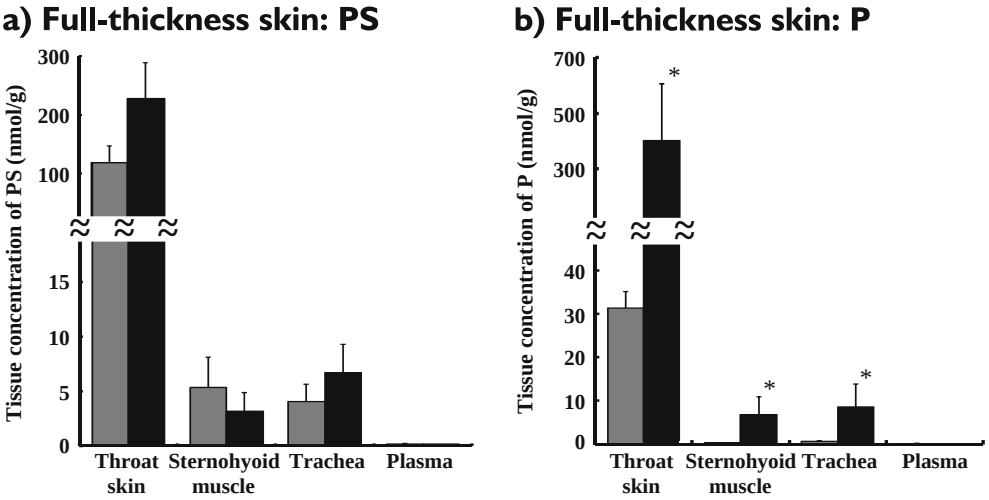


Fig. 7 Amounts of prednisolone succinate (**a**) and prednisolone (**b**) retained in the excised tissue of hairless rats at 8 h after the application of 1% prednisolone sodium succinate solution to full-thickness throat skin. Each point represents the mean ± S.E. ($n = 3–6$). Statistical analysis was performed by Mann-Whitney U-test. *Significant at $P < 0.05$ when compared with non-IP group. Symbols: ▫: non-IP group, ■: IP group.

respectively, and 1674, 10.4, 8.7, and 0.55 nmol/mL for non-IP group, respectively) (Fig. 8a). In addition, little difference was observed in the P concentrations in the stripped throat skin, sternohyoid muscle, trachea, and plasma (372, 5.0, 0.9, and 0.54 nmol/mL for IP group, respectively, and 405, 7.0, 1.3, and 0.63 nmol/mL for non-IP group, respectively) (Fig. 8b).

DISCUSSION

Petelenz *et al.* reported that electrorepulsion from a cathode was more effective than electroosmosis from an anode at delivering the anionic drug dexamethasone phosphate through the skin under the same mA·min value (25), suggesting that the present direct delivery of an anionic drug, FL, through the skin (Fig. 3) was probably due to the electrorepulsion effect from the cathode compartment. The sternohyoid muscle/throat skin FL concentration ratio in the IP group (0.004) was almost the same as that in the non-IP group, whereas the trachea/sternohyoid muscle concentration ratio in the IP group (3.74) was 1.7 times higher than that in the non-IP group (2.19). Thus, the effect of electrorepulsion on FL delivery by cathodal IP treatment was strongly related to the enhanced delivery of FL from the sternohyoid muscle to the deeper tissues such as the trachea. The higher accumulation of FL in the trachea than the sternohyoid muscle both with IP and non-IP groups might depend on the higher affinity of FL to the trachea than the muscle. Thus, the accumulation of FL in the trachea must be due to its direct delivery from the skin surface by IP treatment (Fig. 3).

The enhanced permeation of the anionic prodrug PS through full-thickness skin induced by cathodal IP (Fig. 5) is also explained by electrorepulsion (25–27). The enhanced skin permeation of the prodrug dehydroepiandrosterone induced by anodal IP reported by Laneri *et al.* (28) is similar to the presently observed results. Since cathodal IP treatment increased the skin permeation of PS, the amount of its metabolite, P, in the receiver solution was also

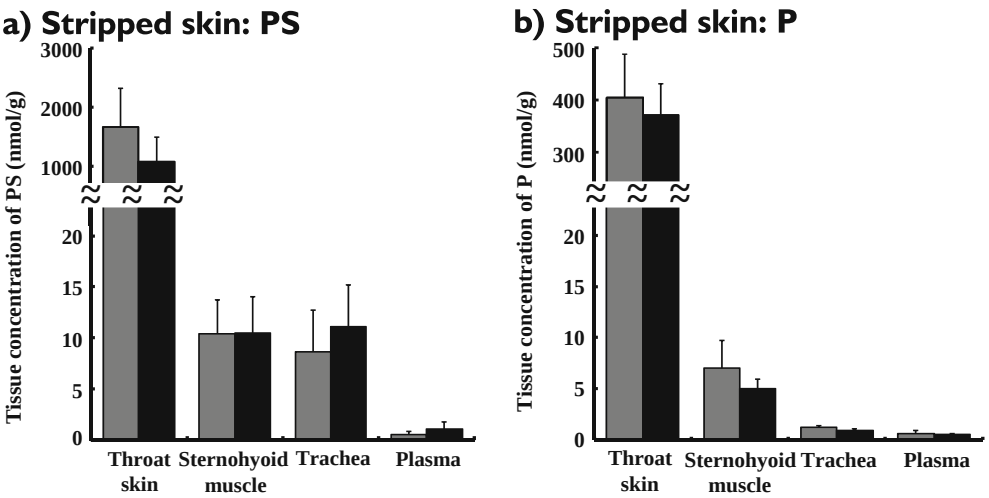


Fig. 8 Amounts of prednisolone succinate (**a**) and prednisolone (**b**) retained in the excised tissue of hairless rats 8 h after the application of 1% prednisolone sodium succinate solution to stripped throat skin. Each point represents the mean ± S.E. ($n = 3–6$). Statistical analysis was performed by Mann-Whitney U-test. Symbols: ▫: non-IP group, ■: IP group.

increased. P was previously found to have a high affinity for skin, resulting in its accumulation in the epidermis (29). This result corresponded to the present data shown in Table 2. On the other hand, IP was not so effective at enhancing the skin permeation of PS and P through stripped skin (Fig. 6). This was due to the higher permeability of stripped skin compared with full-thickness skin (~100 times difference). Esterase activity may be saturated when a high concentration of PS is found in the epidermis (Table 2, Fig. 6). Since the cumulative amount of P passing through full-thickness skin in the IP group was higher than that in the non-IP group, the enhanced permeation of PS induced by IP treatment would have increased the metabolism of PS to P in the skin. The use of IP increased the PS metabolism ratio, P/(PS +P), and decreased the PS concentration ratio, PS/(PS +P), in skin.

The concentration ratio of PS in the trachea/sternohyoid muscle, 2.1, after the application of PS-Na to full-thickness skin and IP treatment was 2.8 times higher than that observed (0.76) without IP treatment. The delivery of PS and P to the trachea was enhanced by IP treatment in a similar manner to FL delivery (Fig. 7a). The marked increases in the P concentrations in the sternohyoid muscle and trachea and the decrease in the P concentration in plasma were probably due to the increased skin permeation of PS and P (Fig. 7b). In contrast, no difference was observed in PS+P delivery through stripped skin to the trachea with or without IP treatment (Fig. 8). Similar results were also observed in the *in vitro* skin permeation experiments. Steroids are usually combined to keratin in skin. Abnormal keratin shows a lower affinity to steroids than normal keratin, resulting in a lower accumulation of steroids in diseased skin than healthy skin (30). In addition, atopic dermatitis patients usually display low skin barrier function, as is seen in stripped skin. Consequently, a high level of direct permeation of PS and P into the trachea probably occurs in such patients.

Although IP is utilized to increase the skin permeation of compounds, it is not always useful for increasing the skin concentration of some compounds. However, high skin permeation of a prodrug such as PS leads to a high concentration of the active drug, such as P, in the skin, resulting in the delivery of a high level of the active drug to deeper tissues, such as the trachea, as explained in Fig. 9. Figure 9 shows schematic illustration for tissue and blood concentrations of PS and P with or without IP application, which are shown in Fig. 7. Thus, IP can be used to increase the concentrations of prodrug metabolites in the skin (IP or non-IP: 401 and 31.4 nmol/g, respectively) and deeper tissues (IP or non-IP: 8.5 and 0.7 nmol/g, respectively) after the application of the prodrug.

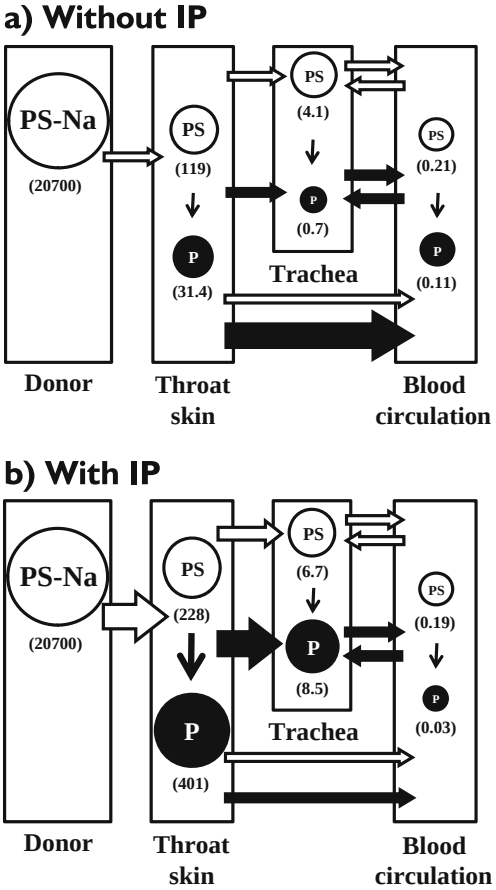


Fig. 9 Schematic illustration of prednisolone sodium succinate or prednisolone migration into the trachea through the throat skin with or without cathodal iontophoresis. The open big arrows show the permeation rate of PS, the closed big arrows show the permeation rate of P, and the small arrows indicate the rate of metabolism of PS to P. The open and closed circles represent the amounts of PS and P (nmol/g), respectively.

CONCLUSION

The possibility of direct delivery of steroids through the skin to the trachea and the effect of IP on the enhanced delivery of the drug through the skin to the trachea were evaluated using *in vitro* and *in vivo* skin permeation experiments. The effect of cathodal IP on the direct delivery of steroids through the skin to the trachea was visually proved using the fluorescent acidic compound FL. IP could increase skin permeation of ionized drugs, but it is not always applicable to increase the skin concentration of applied drugs. We next utilized an acidic steroidal prodrug, PS, to evaluate the direct delivery of the compound and its metabolite, P, to the trachea through throat skin. As a result, we found that IP increased the direct delivery of P to the trachea. This was due to the higher skin permeation of PS as well as the metabolism of PS to P in the skin, resulting in a high trachea concentration of P. Thus, the topical application of

PS to the throat skin with cathodal IP treatment is useful for directly delivering the steroid, P, into the trachea. In the present study, we performed continuous IP application to evaluate the possibility of direct delivery of PS and P to the trachea through the throat skin. Further investigations will be need to elucidate the effect of exposure time and the current density of IP on their direct delivery to the trachea. This drug delivery system may be utilized in asthma therapy for patients suffering from inflammation of the trachea.

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