

Delivery of nalbuphine and its prodrugs across skin by passive diffusion and iontophoresis

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Abstract

The *in vitro* transport of nalbuphine (NA) and its prodrugs across various skins was investigated in order to assess the effects of prodrug lipophilicity on passive as well as iontophoretic permeation. The passive diffusion of NA and its prodrugs increased with the drug lipophilicity. Iontophoresis significantly increased the transport of NA and its prodrugs; the enhancement ratio was highest for NA and decreased as the drug lipophilicity increased. Measurements using intact and stratum corneum (SC)-stripped skins showed that the SC was the major skin diffusion barrier for the passive permeation of NA and nalbuphine pivalate (NAP). The iontophoretic permeation of NA and NAP across intact and SC-stripped skins indicated that the SC layer was not rate-limiting for the permeation of NA, but remained the rate-limiting barrier for transdermal permeation of NAP. Permeation studies using SC-stripped and delipidized skins suggested that the intercellular pathway was the predominant route for the passive permeation of NA and NAP as well as the iontophoretic permeation of NAP across the SC. The relative rates of passive and iontophoretic permeation across Wistar rat skins demonstrated that a significant amount of NA may permeate skin via the appendageal routes, whereas NAP permeated predominantly through the lipid matrix. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Several variables may affect the transdermal iontophoretic permeation of drug molecules, including the physicochemical properties of the drug, the vehicle composition, electronic factors and the skin's barrier properties [1–4]. The lipophilicity of the drug can have a significant influence on transdermal

delivery via both passive diffusion and iontophoresis [3–6].

Nalbuphine (NA) is a narcotic analgesic used in the treatment of both acute and chronic pain. It is a potent analgesic with relatively low side effects [7]. Due to its short elimination half-life and low oral bioavailability [8], frequent injection is needed. In order to maintain the blood nalbuphine concentration and to improve the patient compliance and therapeutic effectiveness in pain management, a series of nalbuphine prodrugs have been synthesized, includ-

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ing nalbuphine propionate, nalbuphine ethanate, nalbuphine pivalate and nalbuphine decanoate [9,10]. Various other nalbuphine prodrug formulations, including a biodegradable implant and microspheres, have also been developed [9,10]. The pharmacokinetic and biopharmaceutic characteristics of NA and its prodrugs suggest the practicality of the transdermal route [11]. Nevertheless, due to the various ester side chains of the NA prodrugs, the drug lipophilicity and thus the permeation characteristics can be significantly different. Accordingly, the effects of the lipophilicity of NA and its prodrugs on the transdermal permeation rates and mechanisms have been studied in a systematic way in order to achieve desired delivery rates.

Although there is a large amount of information available on the passive diffusion of prodrugs, no information on the iontophoretic delivery of prodrugs has been developed. In the present study, the first goal was to assess the permeation characteristics of NA and its prodrugs under passive diffusion and iontophoresis conditions in order to demonstrate the influence of drug lipophilicity. The second goal was to utilize various skin membranes as permeation barriers, including hairless mouse skin, stratum corneum (SC)-stripped skin, delipidized skin, furry Wistar rat skin and human skin, to explore the transdermal transport mechanisms of NA and its prodrugs.

2. Materials and methods

2.1. Materials

Nalbuphine hydrochloride (NA) and three prodrugs, nalbuphine pivalate (NAP), nalbuphine enanthate (NAE) and nalbuphine decanoate (NAD), were synthesized and supplied by The National Defense Medical Center (Taipei, Taiwan). All other chemicals and solvents were analytical grade and used as received.

2.2. Measurement of *n*-octanol–water partition coefficient

The partition coefficients of NA and its prodrugs were determined in *n*-octanol–distilled water at

37°C. Drug concentrations in the aqueous phase before and after partition were determined by HPLC as reported previously [9,10]. The partition coefficients were calculated as the ratio of the equilibrium drug concentration in *n*-octanol to the equilibrium drug concentration in distilled water.

2.3. Stability of NA and its prodrugs in buffer

The stability of NA and its prodrugs in pH 4 citrate–phosphate buffer was examined at $37 \pm 1^\circ\text{C}$. The drug solution (10 $\mu\text{g/ml}$) was placed in a cylindrical device (silicone, 2.5 mm I.D.), tightly capped and agitated with a magnetic stirrer at 600 rpm for 24 h. Samples were taken at regular intervals and analyzed by HPLC [9,10].

2.4. Preparation of skin membranes

Female hairless mice (7–9 weeks old) were killed by cervical dislocation and full-thickness skin was excised from the dorsal region. To obtain SC-stripped skin, adhesive tape was applied to the hairless mouse skin with uniform pressure and then removed. This procedure was repeated 20 times. Delipidized skin was prepared by pretreating the hairless mouse skin with 1 ml of chloroform–methanol (2:1, v/v) for 60 min in order to extract the lipid from the SC. The skin of male Wistar rat (6–8 weeks old) was obtained by sacrificing the rat with ether; the hair of its abdominal region was shaved and the full-thickness skin was then excised. Samples of adult human skin (45–55 years old) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the cadaver skin was rinsed with normal saline. The skin was then sealed in a plastic bag with aluminum foil and stored at -20°C [12].

2.5. *In vitro* permeation experiments

In vitro permeation studies were performed using side-by-side glass diffusion cells with citrate–phosphate buffer (8 ml) at pH 4 as the medium. The drug concentration in the donor compartment was 2.8 mM. The receptor medium was pH 7.4 citrate–phosphate buffer (8 ml). The available diffusion area between cells was 0.785 cm^2 . The stirring rate and temperature were kept at 600 rpm and 37°C , respec-

tively. At appropriate intervals, 200- μ l aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh buffer. The amount of NA and its prodrugs was determined by the HPLC method [9,10].

For in vitro iontophoresis experiments, a pair of Ag/AgCl wires with an effective working length of 15 mm was immersed in the buffer solution as electrodes, with the anode at the donor site and the cathode at the receptor site. The anode and cathode were each positioned 3 cm from the edge of skin. The electrodes were connected to a constant current power supplier (Yokogawa, Japan) and the current density was set at 0.3 mA/cm². The other experimental and sampling conditions were similar to those described in the previous paragraph.

The permeation data were analyzed using the following equation:

$$J_{ss} = dQ/(dt \cdot A) = K_p \cdot C_d$$

where J_{ss} is the flux at steady state, Q is the cumulative mass of drug transferred to the receiver compartment, A is the membrane surface area, K_p is the permeability coefficient and C_d is the drug concentration in the donor compartment measured at time $t = 0$. The permeability coefficients as defined by the equation were obtained from the slope of the cumulative amount transported versus time.

3. Results and discussion

3.1. Passive permeation of NA and its prodrugs

The *n*-octanol–water partition coefficients of NA and its prodrugs are listed in Table 1. As expected, the chemical modifications of NA result in higher partition coefficients for prodrugs with longer ester side chains. Prior to performing skin permeation experiments, the stability of the prodrugs was evaluated in a buffer medium for 24 h. Essentially no detectable decomposition products were observed, indicating that the ionized form of the drugs in pH 4 buffer were chemically stable within the 24-h experimental time frame. Preliminary permeation experiments also demonstrated that, within the limit of detection of 1.2 ng/ml of the HPLC assay, no measurable amount of prodrug was obtained in the receptor cell in 24 h. This demonstrates that the prodrugs were completely hydrolyzed during the permeation process. The degradation of prodrugs during the permeation process has been reported in several previous publications [13–16]. Since the epidermis is a metabolically active tissue, this phenomenon can be attributed primarily to the biotransformation and enzymatic degradation of the prodrug within the skin [13–15]. Accordingly, in the present study, the amount of NA (nmol) in the

Table 1
Physicochemical properties and in vitro permeation data of nalbuphine and its prodrugs

Drug		MW ^a (Da)	log P ^b	Flux (nmol/cm ² /h)	K_p ^c ($\times 10^{-3}$ cm/h)	ER ^d
Nalbuphine	Passive	357.46	0.17	1.40 $\pm$ 0.28	0.50 $\pm$ 0.10	–
	Iontophoresis			150.30 $\pm$ 31.34	73.70 $\pm$ 1.20	103.36
Nalbuphine pivalate	Passive	441.59	1.42	7.22 $\pm$ 1.86	2.58 $\pm$ 0.66	–
	Iontophoresis			79.16 $\pm$ 25.49	228.30 $\pm$ 9.11	10.96
Nalbuphine enanthate	Passive	469.62	1.94	27.19 $\pm$ 6.53	12.77 $\pm$ 3.07	–
	Iontophoresis			222.78 $\pm$ 20.05	104.63 $\pm$ 9.42	8.19
Nalbuphine decanoate	Passive	511.70	3.30	51.58 $\pm$ 9.80	53.95 $\pm$ 10.25	–
	Iontophoresis			19.02 $\pm$ 34.52	124.48 $\pm$ 36.11	2.31

^a MW = Molecular weight.

^b P = *n*-Octanol–water partition coefficient.

^c K_p = Permeability coefficient = flux/concentration of drug in the donor vehicle.

^d ER = Enhancement ratio = iontophoretic flux/passive flux. Each value represents the mean $\pm$ S.D. ($n = 3$).

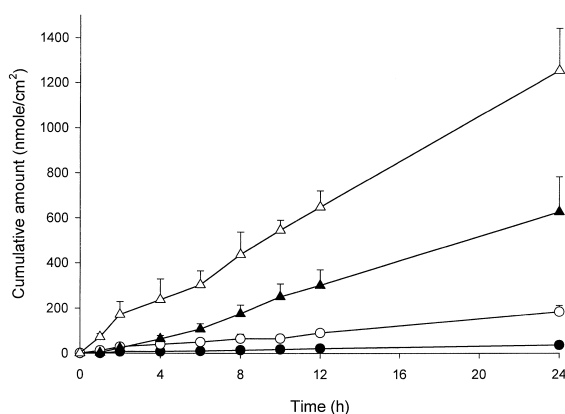


Fig. 1. Cumulative amount of drug permeated per unit area (nmol/cm^2) versus time profiles under passive diffusion: nalbuphine (●), nalbuphine pivalate (○), nalbuphine enanthate (▲) and nalbuphine decanoate (△). All data represent the means of three experiments $\pm$ S.D.

receptor medium was assumed to represent the amount of prodrug permeated across the skin.

Fig. 1 shows the cumulative amount of NA (nmol/cm^2) in the receptor compartment as a function of time for the passive permeation of NA and its prodrugs. The apparent steady-state fluxes (J_{ss}) and permeability coefficients (K_p) obtained from the profiles are summarized in Table 1. Both Fig. 1 and Table 1 show that the permeability coefficients increased with the drug lipophilicity in the order $\text{NAD} > \text{NAE} > \text{NAP} > \text{NA}$. A similar trend has also been observed for the enhanced delivery of mitomycin C across skin using prodrugs [15]. The results demonstrate that, as the chain length of the homologous series increased, the prodrug becomes more effective at delivering the parent compound (NA) across skins. A plot of the permeability coefficients versus partition coefficients is linear with a positive slope of $2.5 \times 10^{-5} \text{ (cm/h)}$ and a correlation coefficient of 0.98. This linear relationship suggests that, as the lipophilicity of the prodrugs increased, more drug molecules partition into the skin membrane, resulting in a higher skin membrane–water partition coefficient and thus a higher skin permeability coefficient. That is, the increased permeability coefficient for the prodrug with longer ester side chain may be attributed to its higher lipophilicity.

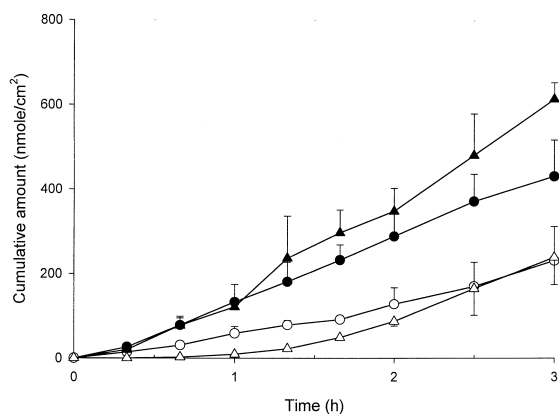


Fig. 2. Cumulative amount of drug permeated per unit area (nmol/cm^2) versus time profiles under iontophoresis: nalbuphine (●), nalbuphine pivalate (○), nalbuphine enanthate (▲) and nalbuphine decanoate (△). All data represent the means of three experiments $\pm$ S.D.

3.2. Iontophoretic permeation of NA and its prodrugs

Fig. 2 shows the amount of drug permeated (nmol/cm^2) versus time for NA and its prodrugs in pH 4 buffer under iontophoresis with a constant current of $0.3 \text{ mA}/\text{cm}^2$. The apparent steady-state fluxes (J_{ss}) and permeability coefficients (K_p) obtained from these plots are listed in Table 1. The data demonstrate that both J_{ss} and K_p increased significantly on application of an external electric field. The trend in the permeability coefficients for the four analogues under iontophoresis condition is different from that under passive permeation, following the order $\text{NAD} > \text{NAE} > \text{NA} > \text{NAP}$. The ratio of the permeability coefficients of the drugs under iontophoresis conditions to the values under passive diffusion, is the enhancement ratio (ER) and is listed in Table 1 for NA and its three prodrugs. The ER for NA was highest (107.36), and decreased as the drug lipophilicity increased, with an ER of only 2.31 for NAD. The results suggest that the application of iontophoresis has a more pronounced enhancement effect on the permeation of the more hydrophilic drug. This observation is consistent with the mechanistic model developed by Kontturi et al. to describe the transdermal transfer of drugs under iontophoresis [6]. In their model, flux was divided into

the contributions through the lipid matrix and through aqueous pores; iontophoresis enhances only the aqueous pathway:

$$J_{if} = J_w^*E + J_o$$

here E is the enhancement factor, J_{if} , J_w and J_o are the flux under iontophoresis, flux through aqueous pathways and flux through the lipid matrix, respectively. According to the simulated results obtained from the model [6], the permeability coefficients of hydrophilic drugs are significantly enhanced by iontophoresis, whereas only a minor increase in permeability coefficients for lipophilic drugs is observed. It can be concluded from the present results and the reported model that the relative importance of lipid matrix and aqueous pores for the four NA analogues under iontophoresis is different, with the more hydrophilic prodrug mainly transported through the aqueous pathway and the more lipophilic drug transported primarily through the lipid matrix of the SC.

The ER values in Table 1 for the four NA analogues may partly be attributed to molecular size. The iontophoretic permeation of ionic solutes has been shown to relate to the molecular volume as well as to molecular weight (MW); higher electrophoretic mobility was observed for the lower MW solutes [17–19]. A correlation coefficient of 0.99 was obtained from the plot of log ER versus log MW, suggesting the molecular size may influence iontophoretic transport. Accordingly, it may be concluded that the different drug lipophilicities, as well as the molecular size of NA and its prodrugs, may contribute to the enhancement effects and thus the ER values.

3.3. Passive permeation of NA and NAP across various skins

Various types of skin were used as barriers in passive and iontophoretic permeation studies to obtain mechanistic information on the transdermal delivery of NA and its prodrugs. Table 2 shows the fluxes and permeability coefficients of NA and NAP across various skins under passive diffusion conditions. Except for the delipidized skin, NAP had higher permeability coefficients than NA for all the

Table 2

The data for in vitro passive permeation of nalbuphine and nalbuphine pivalate across various skins

Drug	Skin types	Flux (nmol/cm ² /h)	K_p^a ($\times 10^{-3}$ cm/h)
Nalbuphine	Hairless mouse	1.40 $\pm$ 0.28	0.50 $\pm$ 0.10
	SC-stripped	57.11 $\pm$ 15.84	20.42 $\pm$ 3.67
	Delipid	54.62 $\pm$ 15.84	19.52 $\pm$ 5.66
	Wistar rat	4.60 $\pm$ 1.10	1.64 $\pm$ 0.39
	Human	1.68 $\pm$ 0.34	0.60 $\pm$ 0.12
Nalbuphine pivalate	Hairless mouse	7.22 $\pm$ 1.86	2.58 $\pm$ 0.66
	SC-stripped	70.60 $\pm$ 4.04	25.24 $\pm$ 1.44
	Delipid	57.58 $\pm$ 16.18	20.59 $\pm$ 5.78
	Wistar rat	8.75 $\pm$ 2.47	3.13 $\pm$ 0.88
	Human	2.94 $\pm$ 1.32	1.05 $\pm$ 0.47

^a K_p = Permeability coefficient = flux/concentration of drug in donor vehicle. Each value represents the mean $\pm$ S.D. ($n=3$).

skin membranes studied. The data in Table 1 also show that the permeability coefficients of NA and NAP transport across SC-stripped skins were 40.79- and 9.18-fold higher than the values across intact skin, suggesting that permeation through the SC layer was the rate-limiting process for the passive permeation of NA and NAP across the skins. The greater enhancement of the permeability coefficients of NA, relative to NAP, after removal of the SC indicates that the SC layer had a more pronounced barrier effect on the more hydrophilic drug.

For the passive permeation of drug molecules through the SC, two routes are involved [20]: (a) permeation through corneocytes (transcellular pathway) and (b) permeation through the lipid bilayer region surrounding corneocytes (intercellular pathway). The relative importance of the two pathways on drug permeation may be evaluated by performing the permeation experiments using the SC-stripped skin and delipidized skin as the barrier membranes. That is, for drugs which permeate mainly through the lipid bilayer region (intercellular pathway), similar permeability coefficients should be observed for those transported through the SC-stripped skin and delipidized skin. Table 2 shows that there is no significant difference in the permeability coefficients (t -test, $P>0.05$) for NA and NAP transported through SC-stripped and delipid skins, suggesting the predominant route for the passive permeation of NA

and NAP across the SC-layer is the intercellular pathway.

Table 2 also shows that the permeability coefficient of NA across furry Wistar rat skin is three times higher than that of NA across hairless mouse skin, whereas no significant difference in permeability coefficients was observed for NAP transport across Wistar rat and hairless mouse skins. This observation indicates the appendageal routes are the important permeation pathways for NA but not NAP. Furthermore, the permeability coefficient of NA across human skin is comparable to that across hairless mouse skin in the present study, suggesting the feasibility of using hairless mouse skin to represent human skin as the barrier membrane in the in vitro passive permeation experiments of NA and its prodrugs in this present study.

3.4. Iontophoretic permeation of NA and NAP across various skins

The factors affecting drug transport through skin by iontophoresis are more complicated than those effecting transported via passive diffusion. The total transdermal permeability of a drug under iontophoresis is determined by the following four contributions: the inherent permeability constant for passive diffusion of the permeant, K_o ; the permeability for the direct effect of the electrochemical potential gradient on charged permeant, $K_{\Delta v}$; the increase in skin permeability induced by the field,

K_s ; and the permeability constant for electroosmotic flow induced by the current, K_{osm} [21,22]:

$$K_{total} = K_o + K_{\Delta v} + K_s \pm K_{osm}$$

The sign of K_{osm} depends on the membrane and electrode polarity. In the present study, the pH of aqueous vehicle used in the donor (pH 4) was approximate equal to the isoelectric point of hairless mouse skin [23]. As a result, the contribution of osmotic flow to drug permeation can be excluded.

Table 3 shows the permeability coefficients of NA across various skins under iontophoresis. No significant difference (t -test, $P > 0.05$) was observed between the permeability coefficients of NA across intact skin and SC-stripped skin of hairless mouse; furthermore, similar permeation coefficients (t -test, $P > 0.05$) were also obtained for the permeation of NA across intact skin and delipidized skins. Those data indicate that the SC layer was not a rate-limiting barrier for the permeation of NA across the skin under iontophoresis. That is, the rate-limiting characteristics of SC layer in the passive transport of NA could be overcome by application of iontophoresis. Since the magnitude of the permeability coefficient for NA across hairless mouse skin via passive diffusion (K_o) is negligible compared to K_{total} (Tables 2 and 3), the overall permeation enhancement of NA under iontophoresis is due primarily to the permeability which arises from the electrochemical potential gradient ($K_{\Delta v}$) and the increase in skin permeability induced by the field (K_s).

Table 3

The data for in vitro iontophoretic permeation of nalbuphine and nalbuphine pivalate across various skins

Drug	Skin types	Flux (nmol/cm ² /h)	K_p^a ($\times 10^{-3}$ cm/h)	ER ^b
Nalbuphine	Hairless mouse	150.30 $\pm$ 31.34	53.70 $\pm$ 11.20	107.36
	SC-stripped	186.77 $\pm$ 25.59	66.99 $\pm$ 9.14	3.27
	Delipid	130.10 $\pm$ 28.97	46.48 $\pm$ 10.35	2.38
	Wistar rat	207.79 $\pm$ 67.06	74.24 $\pm$ 23.96	45.17
	Human	8.37 $\pm$ 2.21	2.99 $\pm$ 0.79	4.98
Nalbuphine pivalate	Hairless mouse	79.16 $\pm$ 25.49	28.30 $\pm$ 9.11	10.96
	SC-stripped	138.57 $\pm$ 28.75	49.54 $\pm$ 10.28	1.96
	Delipid	125.76 $\pm$ 16.10	44.96 $\pm$ 5.76	2.51
	Wistar rat	80.61 $\pm$ 20.68	28.82 $\pm$ 7.39	9.21
	Human	38.06 $\pm$ 16.29	13.61 $\pm$ 5.82	12.95

^a K_p = Permeability coefficient = flux/concentration of drug in donor vehicle.

^b ER = Enhancement ratio = iontophoretic flux/Passive flux. Each value represents the mean $\pm$ S.D. ($n = 3$).

For the permeation of NAP under iontophoresis, however, the permeability coefficient for NAP across the SC-stripped skin was significantly higher than that across intact skin (Table 3). The data again indicate that the SC layer retards the iontophoretic permeation of NAP and that it is a rate-limiting barrier for the permeation of NAP across skins. Moreover, the permeability coefficient of NAP across delipidized skin was comparable to that across SC-stripped skin, suggesting the intercellular lipid matrix but not intracellular corneocytes is the major route for the permeation of NAP across the SC layer during iontophoresis. The above results are consistent with previous reports that iontophoresis may increase the access of ions to the intercellular lipid lamellae [2,24]. By comparing the permeability coefficients of NA and NAP through various skins under iontophoresis, the results clearly demonstrate that different rate-determined processes are involved in the iontophoretic delivery of NA and NAP across skins.

Table 3 shows the permeability coefficients and ER values for drug transport through Wistar rat skin under iontophoresis. The higher permeation coefficient for NA transport through Wistar rat skin relative to hairless mouse skin demonstrates that the transfollicular pathway is more important for the transdermal iontophoretic delivery of NA than for NAP [25]. Table 3 also shows that NA has a higher iontophoretic ER across furry Wistar rat skin than that of NAP (45.17 versus 9.21). Those data suggest that a significant portion of NA may permeate skin via the transfollicular pathway under iontophoresis, whereas NAP permeates predominantly through the lipid matrix. The results are similar to previous findings that the transfollicular route constitutes an important pathway for passive diffusion of NA. Table 3 also shows that the permeability coefficients of NA and NAP across human skin under iontophoresis are much lower compared with those across hairless mouse and Wistar rat skins. This result can be attributed to the fact that the full-thickness human skin was the thickest among all skin types tested in this study. Another explanation is that human skin has 1/10~1/100 less hair follicles than hairless mouse skin [26], resulting in a significant reduction in transport across human skin under iontophoresis.

In summary, the transport characteristics of NA and its prodrugs across various skins by passive diffusion as well as by iontophoresis were examined. In the *in vitro* passive experiments, all prodrugs had a higher permeability than the parent drug (NA). The higher lipophilicity of NA prodrugs, as reflected in the *n*-octanol–water partition coefficients, may result in higher skin–water partition and thus the higher skin permeability. The passive permeation experiments using intact and SC-stripped skins indicated that the SC layer represents the major barrier for the passive permeation of NA and NAP. For the permeation of NA and its prodrugs under iontophoresis, the ER of these four drugs increased with the drug hydrophilicity, confirming previous reports that iontophoresis has a more pronounced enhancement effect on the hydrophilic drugs through the aqueous pathway in the skin. The iontophoretic permeation of NA and NAP through various skins shows that drug hydrophilicity had different rate-determined steps in the permeation process: the SC layer was not rate-limiting for the permeation of NA under iontophoresis, whereas it was rate limiting for the iontophoretic permeation of NAP. The permeation studies using SC-stripped and delipidized skins suggested that the paracellular pathway was the predominant route for the passive permeation of NA and NAP, as well as the iontophoretic permeation of NAP across SC. Both the passive as well as the iontophoretic permeation experiments using Wistar rat skin indicated that the appendageal pathways are the more important routes for the more hydrophilic drug such as NA. The present study demonstrates the feasibility of the transdermal iontophoretic delivery of NA and its prodrugs.

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