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Research paper

Enhancement of transdermal apomorphine delivery with a diester prodrug strategy

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ABSTRACT

Diester prodrugs of apomorphine, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA) were synthesized, and their partition coefficients, capacity factor ($\log K'$), enzymatic hydrolysis, and in vitro permeation across nude mouse skin were characterized. The lipophilicity of the diesters was between that of apomorphine HCl and the apomorphine base. The prodrugs were chemically stable, but enzymatically unstable in esterase medium, skin homogenate, and human plasma. DAA showed a faster hydrolysis in plasma compared to DIA. Total fluxes (nmol/cm^2/h) of the parent drug and prodrug were significantly greater after topical treatment with the diesters in aqueous solutions (water, 30% polyethylene glycol in water, and 30% glycerol in water) compared to treatment with HCl and base forms of apomorphine. DIA flux from deionized water was 51 nmol/cm^2/h, which exceeded the flux of apomorphine HCl by 10-fold. The extent of parent drug regeneration after topical application ranged 51–88% and 34–61% for DAA and DIA, respectively, depending on the vehicles selected. Permeation measurements using intact and stratum corneum-stripped skins demonstrated that the viable epidermis/dermis was an important barrier to prodrug permeation. Nano-sized lipid emulsions were also used as carriers for apomorphine and its prodrugs. Diester prodrugs exhibited superior skin permeation compared to the parent drug when formulated into the emulsions. DAA and DIA fluxes from lipid emulsions were 11- and 3-fold higher than that of apomorphine HCl. The results in the present work suggest the feasibility of diester prodrugs for the transdermal delivery of apomorphine.

1. Introduction

Parkinson's disease affects approximately 1% of people over 65 years old and approximately 3% of those over 85 years old [1]. Apomorphine is considered to be a classical mixed type of dopamine D_1 and D_2 receptor agonists. It is used in the therapy of Parkinson's disease [2]. However, orally administered apomorphine in advanced Parkinson's disease patients is not successful due to the requirement of high doses as a result of metabolic constraints and the first-pass effect. High oral doses of apomorphine can cause gastrointestinal complications and are associated with nephrotoxicity [3]. Apomorphine is most commonly administered by repeated subcutaneous infusions or injections, which invariably result in the appearance of subcutaneous nodules. So far, none of the administration routes has resulted in a delivery system suitable for the widespread clinical application of apomorphine.

Transdermal delivery of apomorphine could be an ideal route of administration due to the advantages of elimination of first-pass metabolism, a reduction in gastrointestinal side effects, and sustained release to resolve the short half-life (32 min) of apomorphine. Although the skin as a route for drug delivery can offer several advantages, the barrier nature of the skin enables only a few molecules to penetrate into and permeate through it. According to the previous study [4], transdermal apomorphine delivery only shows a bioavailability of ~10%. The in vitro permeation of apomorphine by passive diffusion also suggests a negligible flux of near zero [5]. The prodrug approach represents an alternative and very promising method of enhancing skin permeation of drugs. The prodrug concept involves the chemical modification of a drug into a bioreversible form in order to change its pharmaceutical and pharmacokinetic properties and thus enhance its delivery [6,7]. A prodrug strategy to prolong the duration of action in astrocyte

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cultures was reported in the literature [8], but no information exists about the use of apomorphine prodrugs transdermally. The main purpose of this investigation was to develop and evaluate prodrugs for the transdermal delivery of apomorphine. The skin shows high enzymatic activity, mainly due to esterase activity. Ester prodrugs were designed and thoroughly investigated to increase transdermal drug delivery [9,10]. In the present work, two diester prodrugs, diacetyl apomorphine (DAA) and diisobutyryl apomorphine (DIA), were synthesized and evaluated along with apomorphine HCl and apomorphine base. The physicochemical properties and enzymatic hydrolysis of the synthesized compounds were characterized.

The second goal of the study was to utilize nano-sized lipid emulsions as carriers for apomorphine and its derivatives. This work used an in vitro Franz cell to evaluate the skin permeation of apomorphine and the prodrugs using nude mouse skin as the barrier. The contents of both the prodrug and parent drug were determined after transdermal delivery of diester prodrugs into and across the skin. The possible pathways of apomorphine and its prodrugs via the skin were explored to elucidate the transdermal transport mechanisms of the drug/prodrug from lipid emulsions and other vehicles such as water, polyethylene glycol (PEG), glycerol, and mineral oil.

2. Materials and methods

2.1. Materials

Apomorphine HCl, esterase from porcine liver, glycerol, mineral oil, *n*-octanol, and Pluronic F68 were purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA). PEG400 was supplied by Kanto Chemical (Tokyo, Japan). Myverol 18–04 K was obtained from Quest (Naarden, the Netherlands). All other chemicals and solvents were of analytical grade and were used as received.

2.2. Preparation of apomorphine base

Apomorphine base was obtained using a method of precipitation [11]. After a saturated solution of Na_2CO_3 (1.4 g/ml) was added drop-wise to an apomorphine HCl solution in deionized water, apomorphine base was precipitated. The precipitate was then filtered and washed several times with deionized water to remove the Na_2CO_3. After drying, the residual apomorphine base was obtained and verified by infrared (IR) and nuclear magnetic resonance (NMR) analyses.

2.3. Preparation of diester prodrugs

The synthesis, purification, and proof structure of DAA were described previously [12]. The general procedure of the DAA preparation was the use of carboxylic acid anhydride for esterification of apomorphine by utilizing pyridine as the catalyst. This method of DAA to prepare DIA by using isobutyric anhydride in place of acetic anhydride was conveniently extended in this study. Apomorphine hydrochloride (1.00 g, 3.20 mmol), acetic anhydride or isobutyric anhydride (6 ml), and pyridine (1 ml) were mixed in a three-necked round bottom flask with a magnetic stirrer bar under nitrogen. When the mixture was stirred at room temperature for 4 h, the reaction was complete as monitored by thin-layer chromatography. Then, this reaction mixture was diluted by ethyl acetate (70 ml) and a solution of potassium carbonate (7.60 g, 0.055 mol) in water (60 ml). The organic phase was washed by water (20 ml) and brine (10 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a yellow crude oil which was chromatographed through a short column of silica gel to afford DIA (0.99 g, 2.83 mmol) in 88% yield, mp 124–125 °C MS(EI) *m*/*z* (% relative intensity) 266(100), 351(M+, 95), 350(78), 308(70), 265(25), 43(21), 224(21), 352(21), 267(20), 309(17), 206(17). DIA (1.10 g, 2.70 mmol) was also obtained in 84% yield, mp 105–106 °C MS(EI) *m*/*z* (% relative intensity) 266(100), 407(M+, 79), 336(76), 406(61), 43(40), 265(23), 267(21), 408(20), 337(20), 224(17), 294(17).

2.4. Partition coefficients (log P)

A predetermined amount of a methanolic solution of apomorphine or its prodrugs was placed in a glass tube. After completely evaporating the methanol, 1 ml of deionized water and *n*-octanol or mineral oil was added to the tubes. The mixture was shaken reciprocally in an incubator at 32 °C for 24 h. The phases were separated by centrifugation at 5500 rpm for 10 min. The aqueous phase was filtered through a polyvinylidene difluoride (PVDF) membrane with a pore size of 0.45 μm. The drug/prodrug concentrations in both the organic solvent and water were determined by high-performance liquid chromatography (HPLC). Partitioning was calculated as the log $P_{octanol/water}$ or log $P_{MO/water}$ (compound concentration in the *n*-octanol or mineral oil phase/compound concentration in the water phase).

2.5. Capacity factor (log K′)

The K' values of apomorphine and its prodrugs were determined isocratically using HPLC. The HPLC system included a Hitachi L-2130 pump (Tokyo, Japan), a Hitachi L-2200 sample processor, and a Hitachi L-2400 ultraviolet (UV)–visible detector. A 25-cm-long, 4-mm-inner-diameter C18 column (Merck, Darmstadt, Germany) was used. The mobile phase consisted of an acetonitrile: pH 2.5 aqueous solution adjusted with phosphoric acid (20:80) at a flow rate of 1.0 ml/min. The UV wavelength was set to 212 nm. The retention time of each compound was measured, and K' values were calculated using the following equation: $\log K' = \log(t_r - t_0)/t_0$, where t_r is the retention time of each compound and t_0 is the retention time of the non-retained solvent peak (methanol), which was about 1.9 min from the sample injection time.

2.6. Hydrolysis of apomorphine prodrugs

The enzymatic hydrolysis of diester apomorphine was carried out using esterase, nude mouse skin homogenate, and human plasma. The female nude mouse (ICR-Foxn1nu strain, 8 weeks old) was used in accordance with a protocol approved by the Institutional Animal Care and Use Committee at Chang Gung University. After sacrifice, nude mouse skin (at 150 mg) was cut into pieces and placed into a test tube. pH 7.4 buffer (850 μl) was added to the tissue sample and homogenized for 5 min. The homogenate was centrifuged at 9500*g* for 10 min and filtered through a PVDF membrane to obtain the incubation medium. Blood samples were obtained from healthy donors by venipuncture and collected into test tubes containing 124 mM sodium citrate (one volume of sodium citrate solution + nine volumes of blood), which was approved by the Institutional Review Board at Chang Gung Memorial Hospital. The plasma was obtained as described previously [13]. Stock solutions were prepared by dissolving a weighed amount of each prodrug in methanol to give a concentration of 0.5 mg/ml. A volume of 1 ml of this solution was added to the test tube, followed by evaporation of the organic solvent. A volume of 2 ml of esterase (2.02 IU/ml) in pH 7.4 buffer, skin homogenate, or human plasma was added to the test tube. The resulting solution was incubated and shaken at 37 °C. At predetermined intervals, the reaction mixture was withdrawn, and a 400 μl acetonitrile was rapidly added to stop the enzymatic reaction. After thawing and

filtration, the concentration of prodrugs in the medium was measured by HPLC.

2.7. Preparation of lipid emulsions

The oil and aqueous phases were prepared separately. The oil phase consisted of mineral oil (12%, w/v) and Myverol (0.3%), while the aqueous phase consisted of deionized water and Pluronic F68 (2.5%). Apomorphine HCl (to reach a concentration of 1.87 mM in the final product) was positioned in the aqueous phase. On the other hand, apomorphine base or the diester prodrug (1.87 mM) was positioned in the oil phase. The two phases were heated separately to 55 °C for 15 min. The aqueous phase was added to the oil phase and mixed using a high-shear homogenizer (Pro 250, Pro Scientific, Monroe, CT, USA) at 12,000 rpm for 10 min. The mixture was further treated using a probe-type sonicator (VCX 600, Sonics and Materials, Newtown, CT, USA) for 10 min at 35 W.

2.8. Droplet size and surface charge of the lipid emulsions

The mean droplet size (*z*-average) and zeta potential of the lipid emulsions were measured by a laser scattering method (Nano ZS® 90, Malvern, Worcestershire, UK) using a helium–neon laser with a wavelength of 633 nm. Photon correlations of spectroscopic measurements were carried out at a scattering angle of 90°. The zeta potential determination was based on the droplet electrophoretic mobility in aqueous medium. A 1:100 dilution of the systems was made using deionized water before the measurements.

2.9. In vitro transdermal delivery

Skin permeation of apomorphine and its prodrugs was measured using a Franz diffusion cell. Full-thickness dorsal skin of nude mouse was mounted between the donor and receptor compartments. The donor medium was 0.5 ml of deionized water, PEG400/water (3:7), glycerol/water (3:7), mineral oil, or lipid emulsions. The drug/prodrug concentration in the donor compartment was 1.87 mM, which is near the saturated solubility of apomorphine base in water (0.51 mg/ml). The receptor medium consisted of 30% ethanol in pH 7.4 citrate–phosphate buffer for maintaining the sink condition of these permeants, especially the lipophilic apomorphine base and prodrugs. The available diffusion area between compartments was 0.785 cm^2. The stirring rate and temperature were maintained at 600 rpm and 37 °C, respectively. At appropriate intervals, 300 μl of the receptor medium was withdrawn and immediately replaced by an equal volume of fresh buffer. The amounts of apomorphine and its prodrugs were determined by HPLC. The stratum corneum (SC)-stripped skin was also used as the permeation barrier to justify the role of SC on the skin penetration of apomorphine and the derivatives. The skin was stripped 20 times with adhesive tape in the study to obtain SC-stripped skin [14]. The cumulative amounts of the drug/prodrug permeated across the skin as a function of time were profiled. Most of the curves practically matched zero-order kinetics. The linear ascent of the slope of the curves was used to determine the flux (nmol/cm^2/h).

Concentrations of apomorphine and the prodrugs in the skin reservoir were determined after 12 h of transdermal delivery. The skin tissue was rinsed with water and blotted with a paper towel. This procedure could remove the residual drug or prodrug on the skin surface but did not affect the drug/prodrug within the skin. The tissue was weighed and minced with scissors, positioned in a glass homogenizer containing 1 ml methanol, and ground for 5 min with an electric stirrer. The resulting solution was centrifuged for 10 min at 9300*g* and filtered through a PVDF membrane with a pore size of 0.45 μm. The drug/prodrug amount in the supernatant was detected by HPLC.

2.10. Statistical analysis

The statistical analysis of differences among various treatments was performed using unpaired Student's *t*-test. A 0.05 level of probability was taken as the level of significance. An analysis of variance (ANOVA) test was also used if necessary.

3. Results

3.1. Physicochemical characterization of apomorphine and its prodrugs

Apomorphine possesses two hydroxyl groups that provide suitable sites for esterification. The acetyl and isobutyryl moieties were used to form the ester bond for apomorphine as shown in Fig. 1. The melting points of apomorphine, DAA, and DIA are 285, 124, and 105 °C, respectively, according to a previous study [12]. The physicochemical properties of apomorphine and the diester prodrugs are summarized in Table 1. The molecular weight of apomorphine and its prodrugs ranged 267–408 Da, which fell in the appropriate range for transdermal delivery (<500 Da) [15]. The *n*-octanol/water partition coefficients of apomorphine and the prodrugs are listed in Table 1. Apomorphine HCl exhibited an extremely low value for log $P_{octanol/water}$ because no apomorphine HCl was detected in the *n*-octanol phase. Apomorphine base showed a log $P_{octanol/water}$

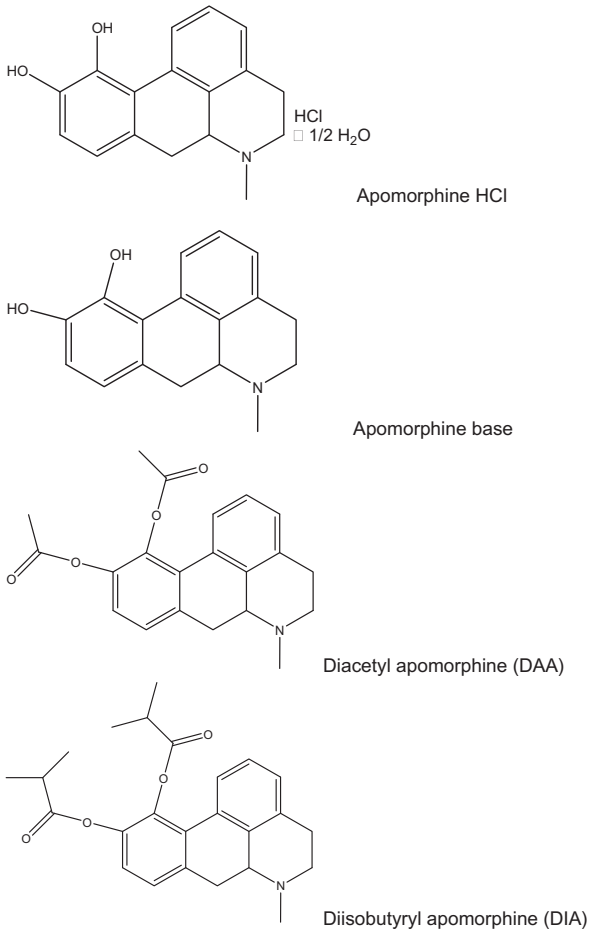

Fig. 1. Chemical structures of apomorphine HCl, apomorphine base, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA).

Table 1
Physicochemical characteristics of apomorphine HCl, apomorphine base, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA).

Compound	Molecular weight (Da)	log $P_{octanol/water}$ [a]	log $P_{MO/water}$ [b]	log K' [c]
Apomorphine HCl	303.8	–[d]	–	0.6 ± 0.01
Apomorphine base	267.3	2.0 ± 0.1	0.5 ± 0.02	0.7 ± 0.01
DAA	351.4	0.8 ± 0.04	0.3 ± 0.03	1.4 ± 0.02
DIA	407.5	1.6 ± 0.03	0.4 ± 0.02	1.8 ± 0.02

[a] log $P_{octanol/water}$, logarithm of octanol/water partition coefficient.
[b] log $P_{MO/water}$, logarithm of mineral oil/water partition coefficient.
[c] log K', logarithm of $t_r - t_0/t_0$, t_r is the retention time of compound, t_0 is the retention time of solvent peak.
[d] –, the compound was completely (100%) existed in water phase.

value of 2. As expected, chemical modification of apomorphine resulted in a higher partition coefficient for prodrugs with longer ester side chains. However, the log $P_{octanol/water}$ of the prodrugs did not surpass the value of apomorphine base. A similar result was observed for the partition coefficient between mineral oil and water (log $P_{MO/water}$). The log $P_{MO/water}$ showed a trend of apomorphine base > DIA > DAA > apomorphine HCl.

The lipophilicity ranking was also evaluated by measuring the capacity factor (log K'), which indicates the relative retention of a compound in the HPLC system. Contrary to the results of the partition coefficients, the capacity factor of apomorphine base was significantly lower ($p < 0.05$) compared to those of the diester prodrugs. The capacity factors of the HCl and base forms of apomorphine were approximately the same ($p > 0.05$).

3.2. Hydrolysis of apomorphine prodrugs

The reconversion of the ester prodrugs into the parent drug is essential for their successful use as transdermal prodrugs. The hydrolysis of diesters in esterase, skin homogenate, and human plasma was determined to examine their susceptibility to enzymatic attack. Porcine liver esterases are regarded as a good model for skin enzymatic activity [6]. Fig. 2A shows the representative stability of prodrugs in esterase medium. The prodrugs were hydrolyzed to the parent drug to a great extent in the presence of esterase. An initial sharp decrease in the prodrug concentration was detected for both DAA and DIA. The prodrugs were completely hydrolyzed by esterase during an incubation period of 60 min. There was no significant difference ($p > 0.05$) between the hydrolysis rates of DAA and DIA. A similar profile of hydrolysis was observed for esterase and skin homogenate (Fig. 2B). No significant difference ($p > 0.05$) was detected in skin homogenate degradation between the two prodrugs except the time point of 20 min, which showed a higher hydrolysis level of DAA compared to DIA. Prodrugs were also treated with human plasma in the same experimental procedure as the esterase treatment (Fig. 2C). The conversion of DAA in plasma was so rapid that no DAA molecules were detected after 40 min. The conversion of DIA by plasma was relatively slower ($p < 0.05$) than that of DAA. DIA showed higher enzymatic stability in plasma than in esterase and skin homogenate. It was apparent that DIA was not completely hydrolyzed throughout the entire experiment. After 120 min, more than 30% of the unmodified DIA prodrug was still present in plasma.

3.3. In vitro transdermal delivery of apomorphine and its prodrugs

Skin permeation of apomorphine HCl, apomorphine base, DAA, and DIA through excised nude mouse skin was evaluated using

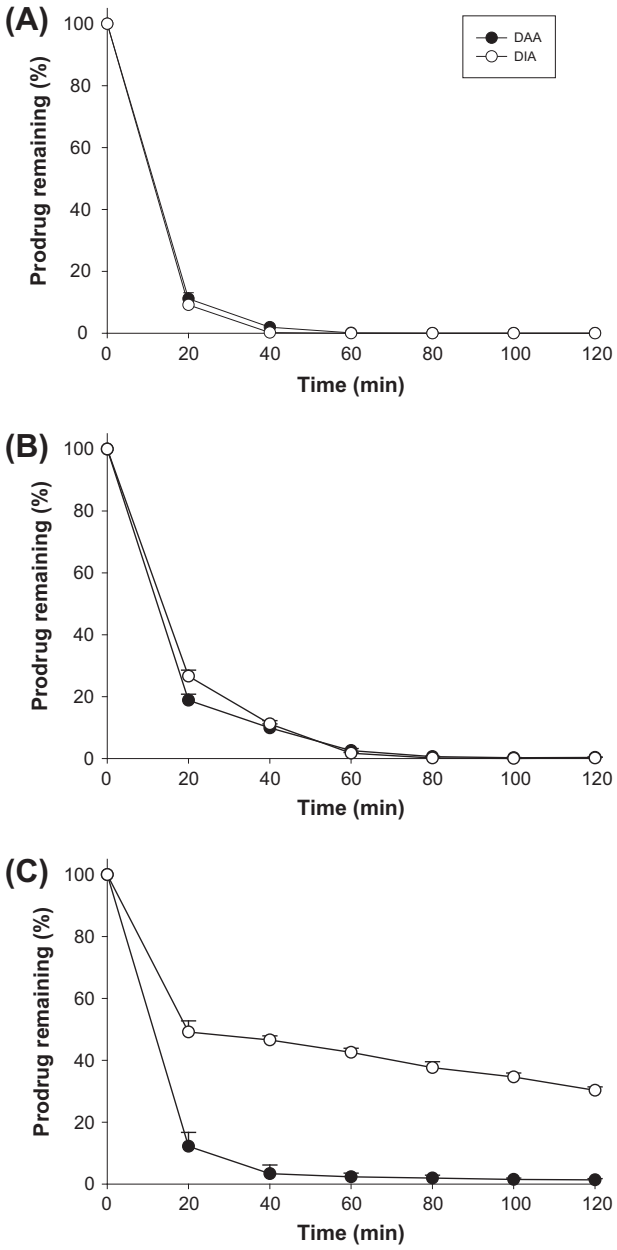

Fig. 2. The hydrolysis of diacetyl apomorphine (DAA) and diisobutyryl apomorphine (DIA) in (A) esterase medium (2.02 IU/ml), (B) skin homogenate, and (C) human plasma at 37 °C. All data represent the mean ± SD of six experiments.

different vehicles, including water, 30% PEG400 in water, 30% glycerol in water, and mineral oil. The vehicles with different environmental polarities were thus included for testing the effect of donor medium on drug/prodrug penetration. Each of the permeants was tested at the same finite dose (1.87 mM). The cumulative amounts of the compounds from water at different times in the receptor medium are shown in Fig. 3 for representative. The flux obtained from the slope of the cumulative amount-time profiles is summarized in Table 2. As can be seen in Fig. 3A, the HCl form of apomorphine showed greater ($p < 0.05$) accumulation in the receptor compared to the base form. In the case of the prodrugs (Fig. 3B), intact ester was found in the receptor phase together with a variable amount of the parent drug. Considering the degradation of prodrugs during skin permeation, the cumulative amounts of both the prodrug and the parent drug were determined and combined in molar units as shown in Table 2. Cumulative amount–time

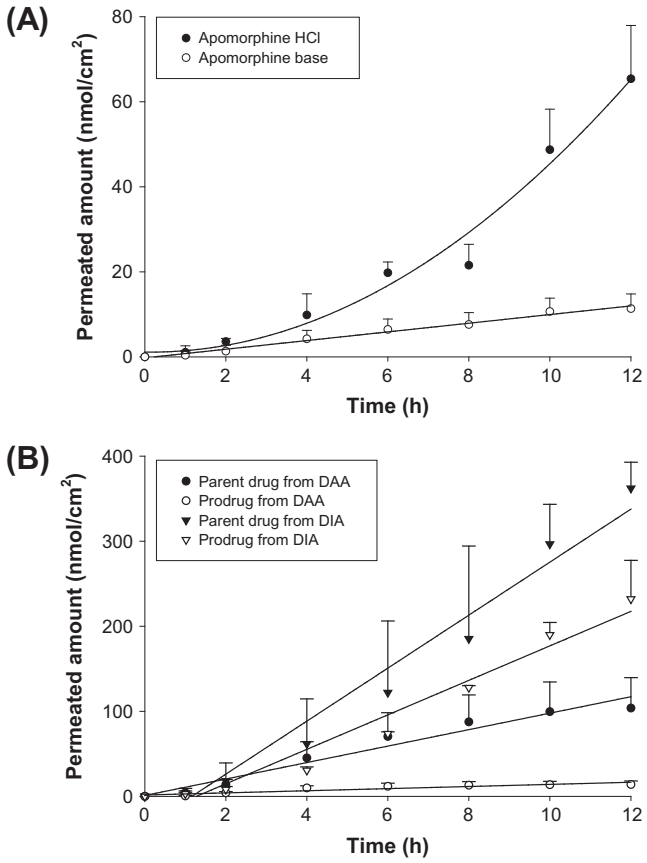

Fig. 3. Permeated amount versus time profiles of the parent drug and prodrug from water by in vitro transdermal delivery of (A) apomorphine HCl, apomorphine base, (B) diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA). All data represent the mean ± SD of four experiments.

profiles demonstrated the attainment of a steady-state flux of apomorphine and the prodrugs, except the HCl form in water (Fig. 3). The steady-state permeation had practically matched zero-order equation. In order to simply compare the permeability of various permeants, the flux of apomorphine HCl from water was calculated by a zero-order fashion although it is untypical.

Skin permeation from water increased in the order of DIA > DAA > apomorphine HCl ⩾ apomorphine base. Due to the low solubility of DIA in water, it appeared to be suspended in the water. The maximum flux of 51 nmol/cm^2/h was achieved for DIA in water, which was 10-fold higher than that of apomorphine HCl. The same trend was observed in vehicles with PEG400 and glycerol. No amount of compound was detected in the receptor for transdermal delivery of apomorphine HCl and base forms from PEG400. Water as the vehicle provided greater permeability ($p < 0.05$) for apomorphine and its prodrugs. Contrary to vehicles with water, DAA showed the highest flux from mineral oil, followed by apomorphine HCl, apomorphine base, and DIA. A significant extent of parent drug regeneration in the receptor was evident in DAA permeation from water and PEG400/water. However, a greater amount of intact prodrug permeated the skin in the case of glycerol/water and mineral oil. The percentages of parent drug in the receptor were 88%, 83%, 56%, and 51% for water, PEG400/water, glycerol/water, and mineral oil, respectively. The levels of parent drug present in the receptor were comparatively low for DIA from water and PEG400/water. The parent drug/prodrug ratios of DIA from glycerol/water and mineral oil were approximately the same as the levels of DAA.

After each permeation experiment, the drugs or prodrugs were extracted from the skin to determine their accumulation and the extent of parent compound regeneration which occurred there. As depicted in Table 3, a significant extent of parent drug regeneration within the skin was evident with DAA delivery. DIA exhibited lower bioconversion in the skin reservoir compared to DAA. The trend of skin deposition of various permeants from different vehicles was generally the same as the results for the flux. Of the homologous series, DIA in the vehicles containing water gave the highest skin accumulation. This prodrug also gave the highest flux through the skin.

3.4. In vitro transdermal delivery across SC-stripped skin

The fluxes of apomorphine and the diester prodrugs permeated via SC-stripped skin are shown in Fig. 4. Deionized water was used as the vehicle for the permeants. An 18-fold increase in flux was detected for apomorphine HCl across SC-stripped skin compared to that across intact skin. The flux of apomorphine base penetrating through SC-stripped skin was more than 2-fold that through intact skin. No significant difference ($p > 0.05$) was seen in the penetration across intact and stripped skin for either prodrug. The ratio of parent drug/prodrug detected in the receptor for stripped skin was the same ($p > 0.05$) as that for intact skin (data not shown).

3.5. In vitro transdermal delivery from lipid emulsions

It is proposed that structural modification of apomorphine, leading to enhanced lipophilicity, may result in feasible nanoparticulate loading. Therefore, utilizing the lipophilic prodrugs and lipid emulsions for further optimum skin delivery of apomorphine was discussed in this study. The lipid emulsion obtained was predominantly composed of mineral oil, Pluronic F68, and Myverol. Results of the size, zeta potential, and polydispersity (PDI) analyses are shown in Table 4. It is evident that the droplet size of lipid emulsions was in the range of nanometer to submicron size (290 nm). Lipid emulsions showed a narrow size distribution

Table 2
Flux (nmol/cm^2/h) of apomorphine HCl, apomorphine base, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA) from various vehicles across nude mouse skin.

Permeant	Compound detected	Water	PEG400/water (3:7)	Glycerol/water (3:7)	Mineral oil
Apomorphine HCl	Apomorphine	5.20 ± 2.57	0	4.80 ± 1.04	16.50 ± 2.77
Apomorphine base	Apomorphine	4.19 ± 1.03	0	0.37 ± 0.19	4.73 ± 1.22
DAA	Apomorphine	9.67 ± 4.43	4.51 ± 0.67	4.20 ± 0.86	17.25 ± 3.10
	DAA	1.25 ± 0.33	0.91 ± 0.14	3.31 ± 0.23	16.29 ± 8.17
	Apomorphine + DAA	10.94 ± 4.69	5.42 ± 0.83	7.52 ± 1.09	33.57 ± 11.31
DIA	Apomorphine	31.16 ± 1.71	11.63 ± 5.93	7.59 ± 3.52	1.02 ± 0.19
	DIA	20.28 ± 3.44	5.97 ± 1.39	4.96 ± 1.52	0.74 ± 0.45
	Apomorphine + DIA	51.46 ± 5.19	17.58 ± 7.30	12.57 ± 5.05	1.78 ± 0.68

Each value represents the mean ± SD ($n = 4$).

Table 3
Skin deposition (nmol/mg) of apomorphine HCl, apomorphine base, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA) from various vehicles across nude mouse skin.

Permeant	Compound detected	Water	PEG400/water (3:7)	Glycerol/water (3:7)	Mineral oil
Apomorphine HCl	Apomorphine	0.30 ± 0.08	0.03 ± 0.04	0.50 ± 0.31	0.92 ± 0.41
Apomorphine base	Apomorphine	0.32 ± 0.03	0.03 ± 0.01	0.12 ± 0.03	0.49 ± 0.15
DAA	Apomorphine	0.56 ± 0.24	0.53 ± 0.22	1.32 ± 0.48	2.11 ± 1.09
	DAA	0.01 ± 0.004	0.03 ± 0.02	0.01 ± 0.004	0.10 ± 0.04
	Apomorphine + DAA	0.59 ± 0.25	0.58 ± 0.23	1.35 ± 0.51	2.34 ± 1.15
DIA	Apomorphine	3.51 ± 1.46	3.31 ± 1.14	1.30 ± 0.53	0.43 ± 0.14
	DIA	1.11 ± 0.33	0.63 ± 0.21	2.58 ± 0.77	0.10 ± 0.02
	Apomorphine + DIA	4.65 ± 1.83	3.96 ± 1.38	3.89 ± 1.33	0.53 ± 0.17

Each value represents the mean ± SD ($n = 4$).

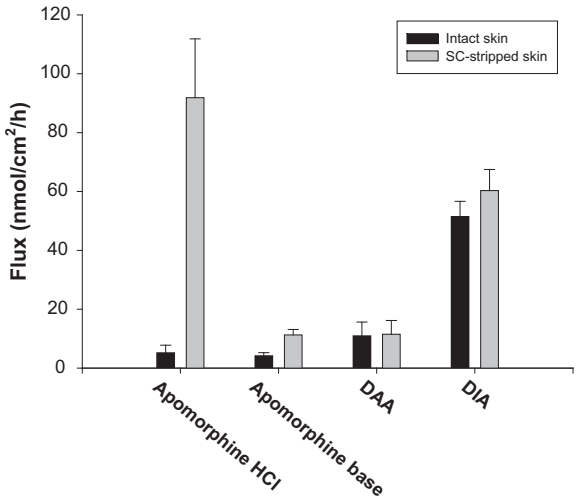

Fig. 4. Comparison of the fluxes (nmol/cm^2/h) of apomorphine HCl, apomorphine base, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA) across intact skin and stratum corneum (SC)-stripped skin. All data represent the mean ± SD of four experiments.

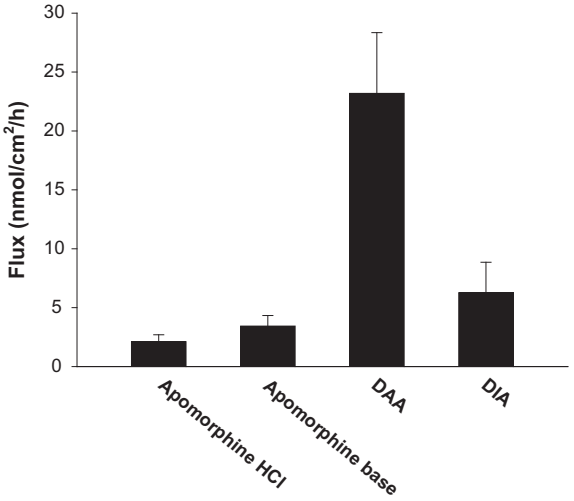

Fig. 5. Fluxes (nmol/cm^2/h) of apomorphine HCl, apomorphine base, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA) from lipid emulsions. All data represent the mean ± SD of four experiments.

(PDI) of 0.15. The zeta potential of the emulsion revealed a negative value of −23 mV.

It was found that the flux of apomorphine HCl was about 2 nmol/cm^2/h for the emulsion as shown in Fig. 5. There was no significant difference ($p > 0.05$) between the flux of the HCl form and base form from the lipid emulsion. For the transdermal delivery of the two diesters, greater permeation was observed for emulsions loaded with DAA. This phenomenon was the same with the prodrug permeation from mineral oil. Following the application of the emulsion, the permeation of DAA increased 11-fold compared to that of apomorphine HCl.

4. Discussion

Clinical failure of most of the potentially effective therapeutics to treat central nervous system (CNS) disorders is often not due to a lack of drug potency but rather shortcomings in the method by which the drug is delivered. The efficient transport of apomorphine is an important step in the onset of the pharmacological activity of this drug. Esters as prodrugs are extensively used because of the simplicity of synthesis coupled with facile enzymatic hydrolysis [16]. We are attempting to develop a transdermal delivery approach for apomorphine. In the present study, the effectiveness of diester prodrugs in enhancing the skin delivery of apomorphine was investigated.

Table 4
The characterization of the lipid emulsion by particle size, polydispersity index (PDI), and zeta potential.

Characteristic	Value
Particle size (nm)	289.70 ± 10.83
Polydispersity index (PDI)	0.15 ± 0.07
Zeta potential (mV)	−22.67 ± 1.05

Each value represents the mean ± SD ($n = 3$).

The partition coefficient is an important factor to describe the skin permeation of a compound. Both prodrugs were more-lipophilic than apomorphine HCl according to log $P_{octanol/water}$ values. The increase in partitioning occurred because the promoiety masked a polar –OH group and the alkyl chains decreased the crystal packing efficiency [17]. However, insertion of the alkyl chain moiety into –OH groups of apomorphine did not increase the lipophilicity as much as with the apomorphine base. The log $P_{octanol/water}$ of DAA was reported to be only 0.8, which is due to the short alkyl chain that is not lipophilic enough to render sufficient lipophilicity. A similar result was observed for quercetin prodrugs with short alkyl chains [10]. The capacity factor of apomorphine derivatives did not show the same trend as the partition coefficients. Apomorphine base with a higher log $P_{octanol/water}$ value demonstrated a lower log K' compared to the prodrugs. This may have been due to the lipophilicity of the compound being influenced by its retention by HPLC, and also the molecular volume and steric structure.

The conversion into parent apomorphine in the enzymatic stability test indicated that DAA and DIA are prodrugs. The esters were effectively metabolized by the esterase in vitro and in the

skin suggesting that the prodrug approach is valid for this system [18]. DAA and DIA demonstrated rapid and comparable hydrolysis in the esterase medium and skin homogenates. Unlike DAA which degraded rapidly, DIA exhibited greater stability in plasma. In general, an increase in the alkyl chain substitution in the ester moiety renders the esters more stable when under enzymatic attack. The bulkier branched ester hydrolyzed more slowly due to the steric hindrance [19,20], which may have reduced contact with enzymes in the plasma. Another possible reason is the effect of prodrug lipophilicity. Most of the enzymes are soluble in the aqueous phase, and for hydrolysis to occur, enzymes and the substrate should be in the same phase in close contact. Thus, the more-lipophilic compounds are more stable because of their low aqueous solubility [21].

The use of nude mouse skin in the present work for the skin permeation test was due to its good accessibility, its common use for in vitro transdermal studies, and its known metabolic activity [22]. Although hairless/nude mouse skin is more permeable than human skin, variations from mouse to mouse are smaller, so that small differences between prodrug/vehicle combinations may be more easily quantified [23]. Also, the rank order of permeation of a homologous series of molecules in hairless/nude mouse skin is the same as it is in human skin, so that in theory, results from mouse skin studies are predictive of results in human skin. In the in vitro permeation experiment, a finite dose of the permeants was used due to the finite dosing more closely resembling an "in use" situation [24]. Although many investigations calculated the permeability coefficient by using a donor compound dose at a saturated concentration in the vehicle, it should not be neglected that the flux, i.e., the mass of permeant transported across the skin, is a more therapeutically relevant parameter than the permeability coefficient [25].

The permeation barrier property of the skin is mediated by a series of lipid multilayers segregated within SC interstices, and their lipophilic nature and tortuous, extracellular localization restrict the transport of compounds across the skin. This contributed to the poor permeability of apomorphine HCl as demonstrated in this study. Molecules with a high melting point show strong intermolecular crystalline self-association. Such molecules have little tendency to dissolve in the organic phase and, consequently, their partitioning into the lipoidal barrier phase of the SC is minimal [26]. Apomorphine HCl has positively ionized format pH values of <7.2 [11]. A higher degree of apomorphine ionization in water (pH 6.4) led to the low permeation since ionized species permeate the SC more poorly than do un-ionized species [27]. Another observation is that the cumulative apomorphine HCl via skin was quickly increased at the late stage of in vitro permeation experiment. A permeant should partition from vehicle to the SC, followed by an accumulation in the SC for further passive diffusion. The hydrophilic HCl form may be difficultly partitioned into the SC. Hence, a slow diffusion occurred at the initial stage of permeation. Once a sufficient amount of apomorphine HCl was retained in SC, the diffusion into receptor would be accelerated. Further study is needed to elucidate the expected mechanisms. The diester prodrugs effectively diffused through the skin, and DIA demonstrated profound potency for penetrating through skin, especially in vehicles containing water. Enzymes in the skin were capable of hydrolyzing apomorphine prodrugs to release the parent compound at a certain level. A decrease in the intramolecular hydrogen bonding in the solid state may have resulted in lower melting temperatures of the diesters, thus increasing the passage into the SC phase. The molecular size and lipophilicity are the main determinants of transdermal delivery [26]. The diffusivity of a series of homologous prodrugs should inversely depend on the third root of their molecular volumes according to a previous study by Waranis and Sloan [28], so that the flux should not change much because of changes in the diffusivity of the prodrugs since their molecular weights do not greatly differ.

The increased lipophilicity with the prodrug strategy resulted in higher skin/water partitioning [29], and thus higher DAA and DIA skin permeation rates from vehicles containing water. It can be seen in Fig. 3B that all cumulative amount–time profiles obey the zero-order fashion although the time lag is different among them. This may suggest the different retention duration of the permeants within the SC. DIA exhibited a longer lag when compared to DAA, which is possibly due to the affinity of more-lipophilic DIA to the SC. DIA provided greater permeation than DAA in the case of water, PEG400/water, and glycerol/water media. All permeants tested at a finite dose of 1.87 mM had completely dissolved in the vehicles selected, except for DIA in water. This was expected since the prodrugs with longer alkyl chains have lower aqueous solubility than the parent drug and the prodrugs with short chains. The thermodynamic activity of the expelled DIA molecules increased due to their poor water solubility. Thus, a supersaturated system was created on the skin surface [30]. DIA in supersaturated water will form precipitates at the bottom of the donor phase, on top of the skin, keeping the resultant solution homogenous which may explain the highest flux observed for all compounds. The DIA concentration immediately adjacent to the skin surface should be replenished faster than it is absorbed into the skin.

A decreased transdermal DIA flux from mineral oil was detected compared to DAA. Besides the lipophilicity and supersaturated condition of the prodrugs, a balance between lipid and aqueous solubilities is essential to optimize the flux, especially from a lipophilic vehicle [23]. The permeation of the total species containing the parent drug depends on the solubility of the prodrug in the SC to provide the driving force for permeation according to Fick's law. In turn, the solubility of the prodrug in the first few layers of skin depends on a balance of lipid and aqueous solubilities which mirrors the properties of the lipid and aqueous phases in these layers [31]. DAA may provide optimal physicochemical properties rather because of its adequate lipophilicity and greater solubility in water compared to DIA. Another reason for the poor permeation of DIA in mineral oil was the low partitioning between the skin and mineral oil, since the oil may cause a high affinity to the lipophilic DIA. Thus, the escape tendency of DIA from the oily vehicle was limited.

Unlike in vitro hydrolysis experiment, the prodrugs did not completely hydrolyze to the parent compound with in vitro transdermal delivery. Homogenization of tissue in the in vitro hydrolysis test exposed some enzymes that might not be as readily available to the prodrugs during transit through intact skin [32]. Slower enzymatic hydrolysis during skin permeation was generally observed for DIA compared to DAA. This indicates that a large amount of DIA in the prodrug form was present after passing through the skin. Apomorphine should be targeted to the brain to exert its therapeutic activity. Once the lipophilicity of the drug is increased by the development of a prodrug, it has improved access to the CNS [33]. However, increased lipophilicity alone does not ensure a higher concentration of the parent drug in the target tissue. Bioconversion in the target tissue needs to be rapid and selective enough to compete with elimination and also to ensure that any premature bioconversion of the prodrug is kept to a minimum [7]. DIA might not completely convert to the parent drug during skin permeation, and some intact prodrug could appear in the systemic circulation. Plasma hydrolysis indicated a relatively low bioconversion of DIA in the circulation. The slow enzymatic conversion rate of DIA is a positive attribute for drug targeting to the brain. The investigation demonstrates that the transdermal delivery of the apomorphine prodrug with bulky ester bonds

may be a promising strategy to achieve CNS targeting for therapeutic use.

Our skin permeation results suggested that the partition coefficient plays an important role in determining the permeation ability. An exception was apomorphine base that showed the highest log *P* but the lowest permeation. Highly lipophilic drugs proved to be poor skin permeants because of their unfavorable partitioning from the SC to the viable epidermis [10]. Since stripping of the SC enhanced apomorphine base permeation by 2-fold, the poor partitioning to viable skin was not the sole factor governing the poor delivery. A possible reason is the inherent instability of apomorphine. Oxidation of hydroxyl groups of the catechol moiety to the quinine form in aqueous solution is one of the factors retarding the stability of apomorphine [2,34]. The incorporation of an HCl salt can partially improve the stability problem of apomorphine [35]. Recent results regarding the stabilization of apomorphine via the formation of a diacetyl derivative leading to increased oxidative stability was quite promising [8]. In order to confirm the aqueous stability of apomorphine and its prodrugs, the compounds at 1.87 mM were incubated in deionized water at 37 °C for 12 h, the same duration for in vitro skin permeation experiment. The amount of these compounds remaining in the water was detected by HPLC. The percentages of the compound remaining in water were 91.3% ± 5.3%, 68.4% ± 4.4%, 95.8% ± 7.5%, and 93.9% ± 6.7% for apomorphine HCl, apomorphine base, DAA, and DIA, respectively. The resistance to instability of the HCl form, DAA, and DIA was markedly enhanced compared to the base form. Therefore, it is reasonable to assume that the presence of the parent drug in the receptor medium was due to enzymatic hydrolysis in the skin and was not the result of substantial chemical hydrolysis in the donor or receptor sites. On the other hand, the significant degradation of the base form may contribute to the poor permeation when compared to the HCl form.

For transdermal delivery, the flux is considered a leading parameter. Although an examination of drug/prodrug deposition in the skin reservoir is less advantageous, an understanding of the skin accumulation may be helpful by elucidating the mechanisms involved in skin delivery of apomorphine and its prodrugs. A similar trend was observed between the skin deposition and flux values of the permeants from different vehicles. It was reported that some ester prodrugs show reduced permeation across the skin, which is due to increased binding of the prodrugs to components in the skin [36]. This was not the case in the present work. The higher skin reservoir of the permeants resulted in high release from the skin into the receptor compartment, because of the fast diffusion due to the concentration gradient. A skin depot that slowly and continuously releases into the receptor compartment is needed for sustained permeation of a compound.

The SC is principally lipophilic in nature and far more resistant to polar than non-polar permeants. This speculation is consistent with the permeation profiles of apomorphine HCl with the removal of the SC which largely increased drug permeation in the ionic form. Stripping the SC did not enhance the total prodrug permeation (parent drug + prodrug in the receptor). The percent of parent drug conversion did not change by stripping the SC. Since the viable epidermis is a metabolically active tissue, this layer primarily contributes to the biotransformation and enzymatic degradation of prodrugs within the skin [9]. Removal of the SC did not affect the enzymatic activity of the whole skin. It appears that the SC is not the sole contributor of resistance to prodrug permeation. The skin is composed of a comparatively lipophilic SC and hydrophilic viable skin. Although the prodrugs could easily get into the SC, they might not be able to escape out of the SC into the viable epidermis [6,10]. The poor prodrug dissolution in the aqueous portion of viable tissue could further slow down the bioconversion to the parent drug [37]. That is the reason why the apomorphine prodrugs were not completely hydrolyzed to the parent drug in the skin. The flux of apomorphine base by SC stripping did not increase so much as that of the HCl salt. In addition to the instability of the base form in water, it was suggested that both the SC and viable skin are predominant sources of resistance for the apomorphine base.

Lipid nano-sized emulsions are a priori interesting candidates for the delivery of drugs via the skin. They are well accepted for their ability to incorporate lipophilic drugs, to reduce side effects of various potent drugs, to increase bioavailability, and to prolong pharmacological effects compared to conventional formulations [38,39]. Apomorphine prodrugs may be easy to formulate into lipid emulsions because of their high oil solubility and lipophilicity. A compound that dissolves or is finely dispersed within the lipid matrix of the carrier should facilitate drug dissolution within epidermal lipids, thus enhancing permeability via the skin [40]. The combination of a prodrug and a specially designed delivery system is feasible to achieve the desired properties. Pluronic F68 is a nonionic species. The negative charge shown in the interface of the emulsion is believed to have resulted in the ionization of Myverol. Some free fatty acids derived from the hydrolysis of monoglycerides in Myverol (palmitinic acid monoglycerides) could have occurred, contributing to the negative surface charge in the shell.

Due to the lipophilic matrix of the lipid emulsions, rather lipophilic substances were considered for loading, whereas hydrophilic substances are expected to be poorly loaded onto droplets. According to log $P_{MO/water}$ values, the apomorphine base and prodrugs had higher encapsulation to partition into the oil phase compared to the ionic HCl salt. Apomorphine HCl flux from the emulsions was lower than that from neat water. Because of the lipid matrix of the emulsions, most of the apomorphine HCl would be in the aqueous phase. The oil droplets may retard the skin permeation of the HCl salt in the external phase because of the prolongation of the apomorphine HCl release path length. It was found that the flux of DAA was greater than that of DIA from the lipid emulsion. This trend was opposite to the case in neat water, but the same was the case with the oily vehicle (mineral oil). Generally, the permeant should be released from the oil droplets, followed by dispersion in water phase and partitioning into the SC. The process of release from oil droplets thus could be the rate-limiting step for permeation. DIA showed a lower penetration than DAA from lipid emulsions. A slower DIA release from the carriers because of the affinity/interaction between more-lipophilic DIA molecules and oil can explain this phenomenon. Thus, the mechanisms involving prodrug permeation from the emulsion may be the feasible lipid/aqueous solubility balance. Another mechanism for skin delivery of the emulsions is the large content of prodrugs in the oil droplets which contacted with skin surface could have substantially partitioned from the oil to the SC layers.

Although apomorphine base was mainly located in the oil matrix, the flux from the emulsion was lower than the flux of prodrugs. The instability of the base form is still a concern in the case of lipid emulsions. DAA and DIA in lipid emulsions could provide greater permeation than the parent drug. DAA in mineral oil and DIA in water also gave high permeation to improve the poor diffusion of apomorphine. However, the uncomfortable skin feeling of the oily vehicle and the instability and variability of the supersaturated system may limit their applicability. Nano-sized emulsions present a choice to resolve these problems. Sustained release for the prodrugs was also proposed to prolong the therapeutic efficacy [41]. We did not attempt to establish the detailed mechanisms of prodrug permeation through the skin from lipid emulsions. The mechanisms will be elucidated in further studies.

It was suggested in the present work that the combination of prodrugs and optimal vehicles can improve apomorphine delivery across the skin. The most permeable system was DIA in deionized water, which exhibited a total flux of 51 nmol/cm^2/h. This flux was

10-times greater than that of apomorphine HCl. Iontophoresis was used as an enhancing technique to promote transdermal apomorphine delivery for patients with Parkinson's disease [42]. The in vitro iontophoretic apomorphine permeation via the human SC was evaluated in a previous study [5]. When no electrical current was applied, the flux of passive delivery was zero. Application of an electrical current (500 $\mu A/cm^2$) resulted in enhancement of the delivery to 90 $nmol/cm^2/h$. It should be noted that comparing the results of separate studies is difficult due to differences in the permeation procedures and evaluation methods used. Hence, a comparison among various enhancement methods should be tentative. Nevertheless, prodrugs can be verified as an efficient approach for improving apomorphine skin permeation. This chemical enhancement does not need an additional device for practical treatment. The cost of the prodrug strategy is also low.

5. Conclusions

The results presented here demonstrate for the first time that diester prodrugs are capable of improving the transdermal delivery of apomorphine. Diesters showed that the main requirements needed for transdermal prodrugs are chemical stability, enzymatic lability, and an increase in the in vitro skin permeation. The good skin delivery observed with these prodrugs was most likely attributed to their lipophilicity and moderate aqueous solubility. Selection of a proper vehicle is necessary for the effective transdermal delivery of apomorphine and its prodrugs. Viable skin is the principle barrier to the permeation of the prodrugs but not apomorphine HCl. The combination of lipophilic prodrugs with lipid emulsions could be a feasible carrier for apomorphine permeation. DAA and DIA appeared to be suitable candidates for apomorphine prodrug design. The advantages of the diester prodrugs warrant further investigations of this approach. Further in vivo studies are planned and in progress to evaluate the potential use of transdermal apomorphine prodrug delivery.

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