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Ester prodrugs of morphine improve transdermal drug delivery: a mechanistic study

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Abstract

Two alkyl esters of morphine, morphine propionate (MPR) and morphine enanthate (MEN), were synthesized as potential prodrugs for transdermal delivery. The ester prodrugs could enhance transdermal morphine delivery. The mechanisms of this enhancing effect were elucidated in this study. Both prodrugs were more lipophilic than their parent drug as evaluated by the skin/vehicle partition coefficient (log P) and capacity factor (log K′). The in-vitro skin permeation of morphine and its prodrugs from pH 6 buffer was in the order of MEN > MPR > morphine. MPR and MEN respectively enhanced the transdermal delivery of morphine by 2- and 5-fold. A contrary result was observed when using sesame oil as the vehicle. The prodrugs were stable against chemical hydrolysis in an aqueous solution, but were readily hydrolysed to the parent drug when exposed to skin homogenate and esterase. Approximately 98% MPR and ~75% MEN were converted to morphine in an in-vitro permeation experiment. The viable epidermis/dermis contributed to a significant resistance to the permeation of ester prodrugs. According to the data of skin permeation across ethanol-, α-terpineol-, and oleic acid-pretreated skin, MEN was predominantly transported via lipid bilayer lamellae in the stratum corneum. The intercellular pathway was not important for either morphine or MPR permeation.

Introduction

Morphine is the most widely used opioid analgesic for acute and chronic pain, and is the standard against which new analgesics are measured (Lugo & Kern 2002). Morphine is inefficiently absorbed orally due to first-pass metabolism. In patients with normal renal function the plasma half-life of morphine is 1.4~3.4 h (Lugo & Kern 2002; Cherny 1996). Transdermal drug delivery offers a further improvement in its administration and enables the continuous systemic application of morphine through intact skin, producing constant plasma concentrations (Grond et al 2000). Drug candidates for transdermal delivery should have a suitable molecular weight of around 200–500 Da, appropriate lipophilicity, and high potency (Doh et al 2003). Most drugs such as morphine show unsuitable physicochemical properties for skin permeation. Morphine exhibits the least permeability across the skin among the narcotic analgesics (Roy et al 1994). In this respect, a prodrug approach may be an alternative way to achieve a systemic effect by delivering the drug through the skin. The prodrug concept involves the chemical modification of a known pharmacologically active compound into a bioreversible form, with the aim of changing its pharmaceutical and/or pharmacokinetic characteristics and thereby enhancing its delivery efficacy and therapeutic value (Chan & Li Wan Po 1989). The prodrugs can be metabolized into the parent drugs by the enzymes within the skin (Guy et al 1987). Drustrup et al (1991) have shown that ester prodrugs of morphine can enhance the delivery of parent drug via the skin. The aim of this study was to explore further the mechanisms of this enhancing effect. Two morphine prodrugs respectively with short and long alkyl ester chains, morphine propionate (MPR) and morphine enanthate (MEN), were synthesized (Figure 1). These two prodrugs had different lipophilicity and molecular size. The comparison of the skin permeation of both prodrugs may be helpful to elucidate the mechanisms of prodrug permeation. The physicochemical characteristics of the prodrugs, including the solubility and partition coefficient, were determined. The hydrolysis of the prodrugs in buffer, skin homogenate, esterase and plasma was

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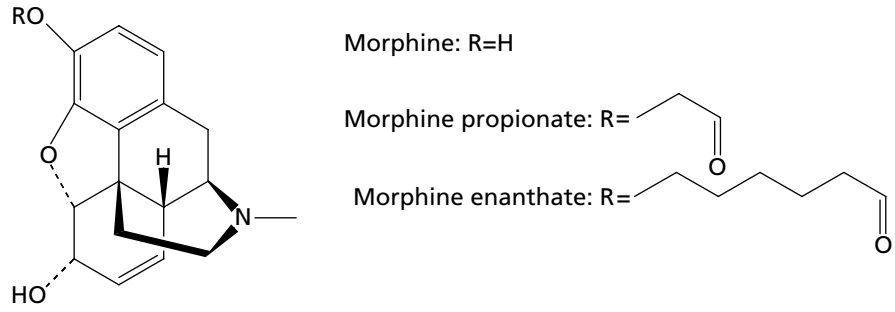

Compound	Molecular weight (Da)	Solubility in pH 6 buffer (mM)	Solubility in sesame oil (mM)	log P[a]	log K′[b]
Morphine	285.3	> 100	0.0221 ± 0.0031	−1.13 ± 0.28	1.96 ± 0.003
Morphine propionate	341.4	30.1 ± 0.2	96.7 ± 4.7	1.75 ± 0.02	11.59 ± 0.24
Morphine enanthate	397.5	3.97 ± 0.1	312.7 ± 9.3	—[c]	145.06 ± 1.00

Figure 1 Chemical structures and physicochemical characteristics of morphine, morphine propionate (MPR) and morphine enanthate (MEN). [a]log P, logarithm of skin/pH 6 buffer partition coefficient, [b]log K′, logarithm of $t_r - t_0/t_0$, t_r is the retention time of product peak, t_0 is the retention time of solvent peak. [c]No compound was detected in pH 6 buffer.

investigated. Both the contents of prodrug and parent drug were determined after transdermal delivery of ester prodrugs into and across the skin. Finally, the possible pathways of morphine and its prodrugs via the skin were explored using skins treated by stratum corneum stripping, α-terpineol and oleic acid to indicate the specific routes for skin permeation of drug/prodrugs.

Materials and Methods

Materials

Sesame oil, α-terpineol, and esterase from porcine liver were purchased from Sigma-Aldrich Chemical (St Louis, MO). Oleic acid was obtained from Wako Chemical (Osaka, Japan). Morphine HCl was supplied by the National Bureau of Controlled Drugs (Taipei, Taiwan). All other chemicals and solvents were of analytical grade and were used as received.

Prodrug synthesis

Morphine HCl (10 g, 35 mmol) was dissolved in 141 mL dichloromethane and triethylamine (8.62 mL, 120 mmol) under argon gas. The solution was placed in an ice bath and stirred. The acid chloride (0.037 mmol) of the desired prodrug moiety was added dropwise. After addition was complete, the reaction mixture was stirred vigorously overnight. Whether the reaction was completed was determined by thin layer chromatography (TLC) with a cosolvent system of ammonium hydroxide:methanol:dichloromethane of 1:6:93. After completion of the reaction, the dichloromethane solvent was dried in a rotary evaporator. An appropriate amount of ethyl acetate was added to the dried product, and transferred to a separatory funnel. The resulting solution was washed with 5% aqueous sodium carbonate and then with water. After evaporation of the organic solvent, the crude product obtained was purified by column chromatography. Purity (>99%) was assured through elemental analysis, nuclear magnetic resonance spectroscopy, and gas chromatography with mass spectroscopy. Two prodrugs, MPR and MEN, were synthesized in this study.

The profiles of MPR were: mp 101–103°C. IR υ^{KBr} cm^{-1} = 3511 (OH), 1753 (C=O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 172.6, 149.2, 134.7, 132.9, 132.6, 132.4, 127.9, 121.6, 120.3, 92.6, 66.2, 59.4, 46.8, 43.3, 42.9, 40.6, 35.4, 27.8, 21.3, 9.5. EI-MS *m/z* (rel. int. %): 341 (M^+, 66), 285 (100), 215 (33), 162 (74), 57 (97).

The profiles of MEN were: mp 79–81°C. IR υ^{KBr} cm^{-1} = 3512 (OH), 1752 (C=O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 171.9, 149.3, 134.8, 132.4 (x 3), 127.7, 121.7, 120.3, 92.6, 66.2, 59.5, 46.9, 43.3, 42.9, 40.5, 35.3, 34.5, 31.8, 29.1, 25.3, 22.9, 21.3, 14.4. EI-MS *m/z* (rel. int. %): 397 (M^+, 22), 285 (100), 267 (18), 215 (16), 162 (38).

Solubility measurement

Excess amounts of morphine and its prodrugs were added to 1 mL of pH 6 citrate–phosphate buffer or sesame oil and shaken reciprocally at 37°C for 24 h. The suspension was centrifuged at 9300 *g* for 10 min, and the supernatant filtered through a PVDF membrane with a pore size of 0.45 μm. The concentrations of morphine and its prodrugs in the filtrate were determined using HPLC after an appropriate dilution. The samples with sesame oil as the medium were diluted using acetone.

HPLC analysis

Morphine and its prodrugs were quantified using an HPLC system consisting of a Hitachi L-7100 pump, a Hitachi L-7200 sample processor, and a Hitachi L-7400 UV detector. A 25-cm long, 4-mm inner diameter stainless C18 column (LiChrospher in LiChrocart column, Merck, Darmstadt, Germany) was used. The mobile phase was a mixture of acetonitrile and a 20 mM phosphate aqueous solution with 1 mM sodium dodecylsulfate at ratios of 32:68, 35:65, and 55:45 for morphine, MPR and MEN, respectively. The flow rate was set at 1 mL min^{-1}, and the wavelength of the UV detector was 212 nm.

Capacity factor (log K′)

The K′ values of morphine and its ester prodrugs were determined isocratically (32% acetonitrile in 20 mM phosphate buffer, 1 mL min^{-1}, UV 212 nm) using HPLC. The retention time of each drug was measured, and K′ values were calculated from the following equation:

$$\log K' = \log (t_r - t_0)/t_0 \quad (1)$$

where t_r is the retention time of each compound and t_0 is the retention time of the non-retained solvent peak (acetonitrile).

Skin/vehicle partition coefficient (log P)

A piece of nude mouse skin (0.7 × 0.7 cm) was positioned in the wells of a 24-well culture plate (17-mm i.d.) with the stratum corneum-side downward. A 100-mL sample 0.2 mM drug in pH 6 buffer was pipetted into the bottom of the well. After 24-h incubation at 37°C, the drug vehicle was withdrawn for analysis by HPLC. The skin/vehicle partition coefficient (log P) was calculated by the following equation:

$$\log P = \log (C_{0\,h} - C_{24\,h})/C_{24\,h} \quad (2)$$

where $C_{0\,h}$ represents the total drug concentration in the vehicle at 0 h before the experiment and $C_{24\,h}$ represents the remaining drug concentration in the vehicle after 24-h incubation.

In-vitro hydrolysis

The in-vitro sensitivity to enzymatic hydrolysis of the prodrugs was carried out using esterase from porcine liver. Esterase (1.92 IU mL^{-1}) in pH 7.4 buffer was sonicated for 3 min before the in-vitro hydrolysis experiments. Each prodrug was dissolved in pH 6 buffer to give a concentration of 0.4 mM. A solution of esterase with a volume of 25 μL was added to 500 μL of the prodrug solution. The mixture was incubated at 37 °C. At predetermined intervals, the reaction mixture was withdrawn and immediately determined by HPLC.

In-vitro transdermal drug delivery

In-vitro skin permeation was evaluated using a Franz diffusion cell. The animal experiment protocol was received and approved by the Institutional Animal Care and Use Committee of Chang Gung University. The dorsal skin of female nude mice (6–8-weeks old) was used as the skin barrier. The donor medium was 1 mL pH 6 buffer or sesame oil, and the drug concentration in the donor compartment was 4 mM. The receptor medium was 5.5 mL pH 7.4 citrate–phosphate (McIlvaine) buffer. The available diffusion area between cells was 1.13 cm^2. The stirring rate and temperature were maintained at 600 rev min^{-1} and 37°C, respectively. At appropriate intervals, 200-μL receptor medium was withdrawn and immediately replaced by an equal volume of fresh buffer. The amounts of morphine and its prodrugs were determined by HPLC. The skin was stripped 20 times with adhesive tape or pretreated by permeation enhancers at a concentration of 3% in 25% ethanol for 2 h if necessary.

The concentrations of morphine and its prodrugs in the skin were determined after 36 h. The skin tissue was rinsed with double-distilled water and blotted with a paper towel. This procedure could remove the residual drug/prodrugs on the skin surface but did not affect the drug/prodrugs within the skin. The tissue was weighed and minced with scissors, positioned in a glass homogenizer containing 1 mL 0.1 M HCl, 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 amount in the supernatant was determined by HPLC.

Statistical analysis

Statistical analysis of differences between the various treatments was performed using the Kruskal–Wallis test. The post hoc test used for checking individual differences between the formulations was Dunn's test. A 0.05 level of probability ($P < 0.05$) was taken as the level of significance. Data entry and analysis was completed using SPSS version 11.5 statistical package program.

Results

Physicochemical characteristics

MPR and MEN represent morphine prodrugs with short (C3) and long (C7) alkyl chain lengths, respectively. It could be expected that morphine, MPR and MEN would show different physicochemical properties such as molecular size, melting point and lipophilicity. These characteristics may influence skin permeation. The appearance of the prodrugs was less crystalline than morphine. This may have been due to a decrease in the intramolecular hydrogen bonding in the solid state, which may have resulted in lower melting temperatures of the esters. This could be confirmed by Drustrup et al (1991), who reported that crystalline behaviour could be strongly depressed by esterification of morphine. The physicochemical properties of morphine and its prodrugs are summarized in Figure 1. The molecular weight of morphine and its ester prodrugs ranged from 285 to 397 Da, which fell in the appropriate range for transdermal delivery (Doh et al 2003). Among the compounds examined morphine showed the highest solubility in pH 6 buffer ($P < 0.05$). The ester prodrugs in buffer showed solubility lower than morphine

($P<0.05$). The aqueous solubility of the prodrugs fell off quickly as the chain length increased.

Contrary to the results for aqueous solubility, morphine was almost insoluble in sesame oil (Figure 1), while the morphine prodrugs revealed high solubility in sesame oil. The prodrugs with a longer alkyl chain (MEN) showed increasing oil solubility with an increase in the number of carbons.

Skin/vehicle partition coefficient (log P) and capacity factor (log K′)

The lipophilicity of morphine and its prodrugs was determined as the skin/pH 6 buffer partition coefficient (log P). As shown in Figure 1, log P of the compounds increased as the alkyl ester chain lengthened. MEN showed an extremely large value for log P because no MEN was detected in pH 6 buffer when evaluating log P. 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 (Figure 1). A good linear relationship between log P and log K′ was observed. log K′ of MEN was much higher than those of morphine and MPR ($P<0.05$). Determining log K′ instead of log P may be a more convenient alternative for estimating lipophilicity.

In-vitro hydrolysis

A successful prodrug for transdermal delivery should exhibit optimum lipophilicity and should resist chemical degradation before hydrolysis within the skin by enzymes. As shown in Figure 2, no significant chemical hydrolysis of either of the ester prodrugs in the buffer was noted during the time course of the experiment. This indicated that both prodrugs were highly resistant to chemical hydrolysis. The enzymatic hydrolysis of the prodrugs by esterase from porcine liver was evaluated at 37 °C as shown in Figure 2. The degradation of MPR followed first-order kinetics. The conversion of MEN by esterase was faster than that of MPR. Human plasma was also treated with the prodrugs in the same experimental procedure as the esterase treatment. The conversion of prodrugs in plasma was so rapid that no prodrug was detected immediately after adding the prodrugs to the plasma (data not shown).

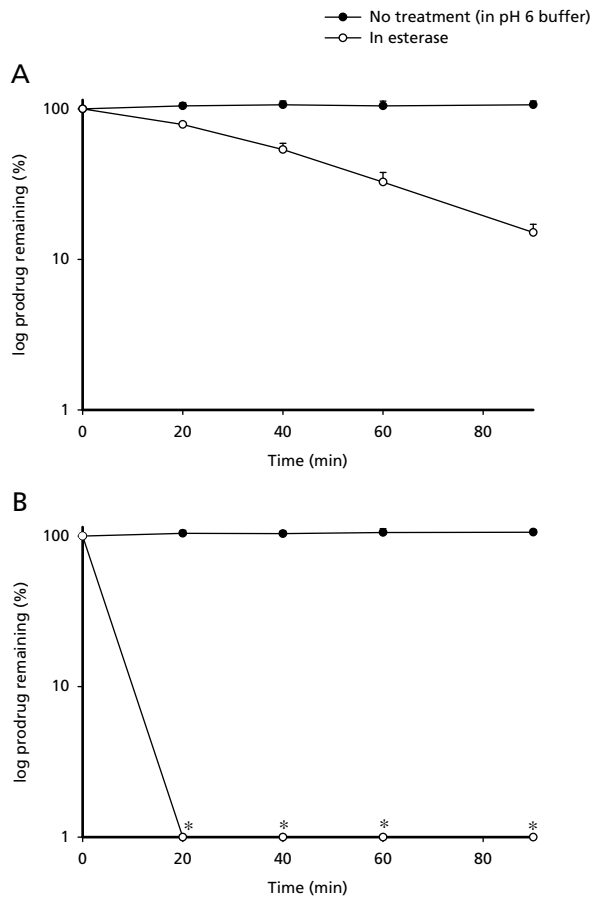

Figure 2 First-order plots of the hydrolysis of morphine propionate (MPR; A) and morphine enanthate (MEN; B) in pH 6 buffer, skin homogenate, and esterase from porcine liver at 37°C. All data represent the mean ± s.d. of four experiments. *$P<0.05$, the degradation value of MEN significantly lower than that of MPR in the presence of esterase.

In-vitro transdermal drug delivery

Permeation of morphine, MPR and MEN through excised nude mouse skin was evaluated using pH 6 citrate–phosphate buffer. Each of the permeants was tested at the same finite dose (4 mM). Morphine and its prodrugs were completely dissolved in this condition according to the aqueous solubility in pH 6 buffer. Figure 3A shows the cumulative amounts of morphine and its prodrugs (nmol cm^{-2}) in the receptor compartment vs time profiles for the permeation from the aqueous buffer. The apparent steady-state fluxes obtained from the profiles are summarized in Table 1. In the case of the prodrugs, intact ester was found in the receptor phase together with a variable amount of the parent drug. MPR was quickly hydrolysed in the skin, and mainly morphine (~98%) was found in the receptor compartment, while ~25% of the intact prodrug remained in the receptor phase after transdermal MEN delivery. Drug permeation was calculated as the sum of the prodrug and morphine as depicted in Table 1. Skin permeation from the aqueous buffer increased in the order of MEN > MPR > morphine ($P<0.05$). The MEN prodrug even increased the total morphine equivalent delivery rate by a factor fivefold greater than the delivery of morphine itself. After each permeation experiment, the drugs were extracted from the skin to determine their accumulation and the extent of parent drug regeneration which occurred there (Table 1). A significant extent of parent drug regeneration in the skin was evident with MPR delivery. On the other hand, the extents of the prodrug and parent drug within the skin were comparable ($P>0.05$) with MEN. The total morphine equivalent skin deposition of MEN was significantly higher ($P<0.05$) than those of morphine and MPR.

The total equivalent morphine flux and skin deposition of morphine and its prodrugs from sesame oil showed a contrary trend to the results from the aqueous buffer (Figure 3B and Table 2). Proportions of the parent drug within and across the skin for MEN were lower ($P<0.05$) than those for MPR when sesame oil was used, which showed the same trend with the aqueous vehicle. No parent drug was detected in the donor phases of either pH 6 buffer or sesame oil during a 36-h permeation period, indicating an acceptable stability for the prodrugs.

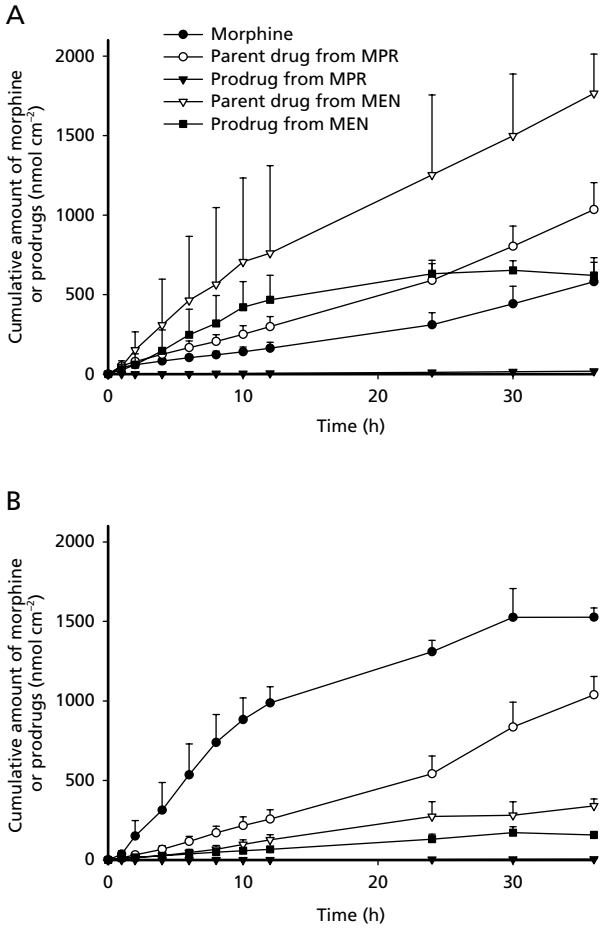

Figure 3 Cumulative amount vs time profiles for the parent drug and ester prodrugs after application of morphine, morphine propionate (MPR) and morphine enanthate (MEN) for permeation across nude mouse skin using pH 6 buffer (A) or sesame oil (B). All data represent the mean ± s.d. of four experiments.

Permeation of morphine and its prodrugs across various skin types

The flux and skin deposition of morphine and its prodrugs permeated via stratum corneum-stripped skin are shown in Table 3. The data indicated that the morphine flux across stratum corneum-stripped skin was 3.64-fold higher ($P < 0.05$) than the flux across intact skin. MPR showed a 2.51-fold increase ($P < 0.05$) in the total morphine equivalent flux after stripping the stratum corneum. The removal of the stratum corneum decreased the delivery of MEN ($P < 0.05$) across the skin. Moreover, the stratum corneum-stripped skin was able to convert the MEN prodrug into the parent drug to a significant level of nearly 100%. This effect was not observed in the case of intact skin. The skin deposition of morphine and its prodrugs was lower in stratum corneum-stripped skin compared with intact skin, which may have been due to the loss of the stratum corneum as a skin reservoir of drugs. To explore further the permeation mechanisms of morphine and its ester prodrugs, α-terpineol and oleic acid were used as permeation barriers to pretreat the skin. Ethanol (at 25%) was used as the pretreatment medium for α-terpineol and oleic acid for solubility considerations. Permeation data across enhancer-pretreated skin are summarized in Table 4. The prodrug/parent drug ratio of MPR and MEN across the pretreated skin was similar to that across intact skin. To simplify the comparison, only the total permeated amount (i.e. the sum of the intact prodrug and the converted morphine) of each ester prodrug is shown in Table 4. The results show that pretreatment with 25% ethanol on the skin reduced the permeation of morphine and its prodrugs. The enhancement ratio by enhancers was calculated. This value was normalized by the flux from 25% ethanol to neglect the vehicle influence. Pretreatment with the permeation enhancer of 25% ethanol increased the permeation of all drugs examined in this study ($P < 0.05$). The enhancement decreased following an increase in drug lipophilicity. The permeation enhancement for morphine and its prodrugs by α-terpineol was slightly higher than that by oleic acid.

Discussion

Nude/hairless mouse skin was used as a model for skin permeation due to its good accessibility, its common use for in-vitro transdermal studies, and its known metabolic activity (Lipp et al 1998). It cannot be neglected that porcine skin and rhesus monkey skin are the best substitutes for human skin (Wester et al 1980, 1983; Riviere & Papich 2001). Although nude mouse skin is more permeable than human skin, variations from

Table 1 Flux and skin deposition of morphine and its prodrugs from pH 6 buffer across intact nude mouse skin

Compound	Compound determined	Flux (nmol/cm^2/h)	Skin deposition (nmol mg^{-1})
Morphine	Morphine	14.61 ± 4.08	1.09 ± 0.66
Morphine propionate (MPR)	Morphine	27.06 ± 4.45	0.69 ± 0.12
	MPR	0.51 ± 0.10	0.01 ± 0.004
	Morphine + MPR	27.57 ± 4.57*	0.70 ± 0.12
Morphine enanthate (MEN)	Morphine	53.97 ± 4.20	1.67 ± 0.45
	MEN	18.48 ± 3.37	1.74 ± 0.71
	Morphine + MEN	72.45 ± 7.59*	3.41 ± 1.16*

Each value represents the mean ± s.d. (n = 4). *$P < 0.05$, the equivalent flux or skin deposition value of prodrugs significantly higher than that of morphine by Dunn's test.

Table 2 Flux and skin deposition of morphine and its prodrugs from sesame oil across intact nude mouse skin

Compound	Compound determined	Flux (nmol/cm^2/h)	Skin deposition (nmol mg^{-1})
Morphine	Morphine	43.65 ± 1.78	0.40 ± 0.15
Morphine propionate (MPR)	Morphine	28.37 ± 4.09	0.56 ± 0.29
	MPR	0.15 ± 0.11	0.01 ± 0.005
	Morphine + MPR	28.52 ± 4.23*	0.57 ± 0.31
Morphine enanthate (MEN)	Morphine	7.04 ± 1.25	0.23 ± 0.08
	MEN	5.62 ± 1.17	0.16 ± 0.06
	Morphine + MEN	12.66 ± 2.43*	0.40 ± 0.16

Each value represents the mean ± s.d. (n = 4). *$P < 0.05$, the equivalent flux or skin deposition value of prodrugs significantly lower than that of morphine by Dunn's test.

Table 3 Flux and skin deposition of morphine and its prodrugs from pH 6 buffer across stratum corneum-stripped skin

Compound	Compound determined	Flux (nmol/cm^2/h)	ER_{Flux}[a]	Skin deposition (nmol mg^{-1})
Morphine	Morphine	53.15 ± 3.46	3.64	0.31 ± 0.10
Morphine propionate (MPR)	Morphine	68.41 ± 1.22	2.52	0.39 ± 0.15
	MPR	0.79 ± 0.13	1.55	0.009 ± 0.001
	Morphine + MPR	69.20 ± 0.38*	2.51	0.40 ± 0.15
Morphine enanthate (MEN)	Morphine	49.79 ± 3.59	0.92	1.23 ± 0.14
	MEN	0.22 ± 0.01	0.01	0.10 ± 0.02
	Morphine + MEN	50.01 ± 3.61	0.69	1.33 ± 0.18*

[a]ER_{Flux}, enhancement ratio = flux across SC-stripped skin/flux across intact skin. Each value represents the mean ± s.d. (n = 4). *$P < 0.05$ the equivalent flux or skin deposition value of prodrugs significantly higher than that of morphine by Dunn's test.

Table 4 Flux and skin deposition of morphine and its prodrugs from pH 6 buffer across the skin pretreated by enhancers

Compound	Enhancer	Flux (nmol/cm^2/h)	ER_{Flux}
Morphine	25% EtOH	5.99 ± 1.18	—
	α-Terpineol/25% EtOH	38.14 ± 7.08*	6.37
	Oleic acid/25% EtOH	31.53 ± 5.65*	5.26
MPR (parent + prodrugs)	25% EtOH	11.06 ± 5.96	—
	α-Terpineol/25% EtOH	55.42 ± 13.18*	5.01
	Oleic acid/25% EtOH	35.41 ± 6.61*	3.20
MEN (parent + prodrugs)	25% EtOH	46.15 ± 18.76	—
	α-Terpineol/25% EtOH	61.17 ± 3.54	1.33
	Oleic acid/25% EtOH	52.56 ± 5.33	1.14

[a]ER_{Flux}, enhancement ratio = flux treated by enhancer in 25% EtOH/flux treated by 25% EtOH. Each value represents the mean ± s.d. (n = 4). *$P < 0.05$, the equivalent flux of morphine and the prodrugs treated with enhancers significantly higher than that treated with no enhancers (25% ethanol as the vehicle) by Dunn's test.

mouse to mouse are smaller, so that small differences between prodrug/vehicle combinations may be more easily quantitated (Sloan & Wasdo 2003). Thus the need for large numbers of diffusion cells for each prodrug/vehicle combination and the need for control diffusion cells per each different human skin sample is obviated. Also, the rank order of permeation of homologous series of molecules in nude/hairless mouse skin is the same as it is in human skin (Scheuplein & Blank 1973; Sloan et al 1997), so that in theory, results from mouse skin studies can be predictive of results in human skin (Sloan & Wasdo 2003). The stratum corneum is generally believed to be the main barrier to skin permeation processes. The horny layer is basically a lipophilic barrier, and so drug lipophilicity is regarded as one of the key parameters controlling drug permeation. One of the main objectives in transdermal prodrug design is to obtain prodrugs with increased lipophilicity compared with the parent drug. It was outlined how more lipophilic drug derivatives show better partitioning and solubility into the stratum corneum, which can result in enhanced skin permeation (Bonina et al 2002). This can be explained by considering that the partition coefficient, which is correlated to measured values of log P and log K′, has a fundamental role in determining the permeability across the skin. The log P between skin and vehicle is a more important factor to relate with the skin permeation of a drug rather than the partition coefficient between n-octanol and buffer. The log $P_{\text{n-octanol/pH 7.4 buffer}}$ values are –0.15, 0.66, and 2.04 for morphine, MPR and MEN, respectively (Drustrup et al 1991; Stinchcomb et al 2002). A higher value was obtained for skin partitioning than partitioning between n-octanol and buffer. As shown in Figure 1, lipophilicity parameters of morphine and the prodrugs increased as the alkyl chain lengthened. This was as expected due to the stratum corneum's control of drug permeation.

Besides the influence of lipophilicity, the molecular weight may be another factor affecting drug permeation. The drug flux is equal to the product of the partition coefficient and the diffusivity (D) of the drug and/or prodrug in the membrane divided by the thickness of the membrane, h_s (assumed to be relatively constant). The diffusivity of a series of homologous prodrugs should depend inversely on the third root of their molar volumes (Waranis & Sloan 1987), so that the flux should not change much because of changes in the diffusivity of the prodrugs since their molar volumes are not very different.

An increase in the alkyl ester chain from C3 (MPR) to C7 (MEN) may increase the total morphine equivalent flux from the aqueous buffer by 2.6-fold. This increment is not so large which may be due to the high permeability of mouse skin. The three compounds tested were totally dissolved in the donor. The pK_a value of morphine is ~8.1 (Roy & Flynn 1989). Morphine is predominantly in an ionic form in pH 6 buffer, which is beneficial to aqueous solubilization. The drug dose in the donor vehicle was set at the same level as MEN solubility in pH 6 buffer. Saturated drug in the vehicle can be used to maintain a constant driving force with maximum thermodynamic activity (Doh et al 2003). The thermodynamic activity of a drug is a function of percent saturation in the vehicle. Drugs are in constant motion due to thermodynamic activity and will move by diffusion down a concentration gradient. It is possible that higher thermodynamic activity results in a higher partition coefficient and delivery of drugs into the stratum corneum (Vaddi et al 2005). This inference could be confirmed by the extremely high log P of MEN. Another plausible explanation for the increased morphine flux from the prodrugs is the metabolism in the skin, which causes a difference in the concentration gradient profile of the prodrugs. It is generally believed that most of the enzyme activity resides in the viable epidermis and dermis layers of the skin (Rautio et al 1998; Sung et al 2000). The affinity between prodrugs and the lipophilic stratum corneum may produce a large accumulation of MPR and MEN in the stratum corneum. The ester prodrugs may be quickly converted into the parent drug when penetrating the viable epidermis/dermis. Hydrophilic morphine can pass rapidly across the hydrophilic epidermis/dermis. A steepening of the intact prodrug concentration gradient may contribute to the enhancement of morphine delivery via the skin (Hammell et al 2004). As shown in Figure 2, MEN with a longer alkyl chain resulted in faster metabolism in the in-vitro enzymatic hydrolysis experiment. It was reported (Ahmed et al 1997; Ostacolo et al 2004) that the hydrolysis rate of some esters was enhanced by increasing their lipophilicity. However, the parent drug proportions of MEN into and through the skin were significantly lower ($P < 0.05$) than those of MPR in the in-vitro permeation experiment. Such a discrepancy in enzymatic hydrolysis between esterase and intact skin may have been due to the different conditions of esterases between the two types. Hydrolytic enzymes have a different subcellular distribution in real skin, since there are membrane and cytoplasmic isoforms, and the different lipophilicities of the derivatives can influence the partitioning and/or distribution in different regions (e.g. the membrane, cytoplasm and extracellular fluid) of tissues (Hikima et al 2002; Ostacolo et al 2004). The long alkyl chain in the steric structure of MEN may have reduced its contact with esterases in intact skin, as a more complex condition, resulting in the relatively lower conversion into morphine compared with MPR. No matter what happened in skin tissue, the prodrugs may have been completely converted into the parent drug when they traversed into the systemic circulation because of the fast metabolism in plasma, as indicated in our experiment.

Due to the low solubility of morphine in sesame oil (0.0221 mM), it appeared to be suspended in the oil. The lipophilic prodrugs were completely solubilized in sesame oil. The permeation of a polar parent drug may be greater from a lipoidal vehicle in which it is not very soluble (Waranis & Sloan 1987). As the lipophilicity of the derivatives decreases, the permeants become less like sesame oil and the permeation increases. Gerber et al (2006) indicated that in the supersaturated vehicles the drug/prodrugs of acetylsalicylic acid had crystals at the bottom of the donor phase, on top of the skin, keeping the resultant solution homogenous, which may explain the higher flux observed for these compounds. Drustrup et al (1991) also synthesized morphine prodrugs including MEN (compound IX in their code) for skin permeation. The transdermal permeation of MEN was greater than that of morphine when an oil medium of isopropyl myristate was used as the vehicle. The increased solubility of the esters in the vehicles combined with expected concomitant increase in solubility in the skin was most certainly responsible for the higher flux. This result was contrary to ours. It may suggest that various oil phases can contribute to the various mechanisms for skin permeation of drugs and/or prodrugs. It is our opinion that isopropyl myristate may act as a penetration enhancer to promote drug delivery across skin. Isopropyl myristate can retard the rate of water loss from the skin, resulting in the accumulation of water content in the epidermis and thus the increase of drug permeation across skin (Fang et al 1996).

It was noted that the permeated amount of intact MEN (without bioconversion in the skin) levelled off after transdermal MEN application with the aqueous solution and sesame oil (Figure 2). A possible explanation for this was that the enzymatic hydrolysis in the in-vitro permeation experiment may have been mediated by enzymes leached from the skin and into the receptor. This effect was more significant following an increase in the application time. This also indicated that the esterases were active during the experimental period (36 h). Differences in the physicochemical characteristics of morphine and its prodrugs suggested that the permeation processes for morphine, MPR, and MEN may have differed kinetically and mechanistically. The flux enhancement for morphine and its prodrugs after stripping the stratum corneum decreased as the drug lipophilicity increased (Table 2). Morphine showed the highest enhancement of skin permeation across stratum corneum-stripped skin, suggesting that permeation through the stratum corneum layer was the rate-limiting process for morphine. The prodrug permeation profiles after stratum corneum-stripping suggested that the permeation of MPR and MEN was hindered not only by the stratum corneum, but the viable epidermis/dermis beneath the stratum corneum may have contributed to the aqueous resistance for their diffusion. Any compound possessing such

a high log P would most likely be much too lipophilic to enter viable skin (Roy & Flynn 1989; Lipp et al 1998; Huang et al 2005). MEN even showed reduced permeation after stratum corneum removal. Lipophilic modification to obtain prodrugs can increase partitioning into the stratum corneum, forming a reservoir (Bonina et al 2002). MEN, which easily passed across viable skin, was quickly metabolized to morphine once it had permeated into the epidermis. The loss of the stratum corneum abolished this effect. After removal of the stratum corneum, MEN had to first be partitioned into the epidermis, and then diffusion occurred. This is difficult for the highly lipophilic derivative, resulting in lower permeation of MEN across stratum corneum-stripped skin. The removal of the stratum corneum promoted the conversion of MEN into its parent drug (Table 3). This may have been due to the direct contact between MEN and the epidermis, which hastened the conversion process.

Pretreatment with 25% ethanol significantly reduced drug permeation, especially for morphine and MPR (Table 4). Protein denaturation in the stratum corneum may have been involved in this reduced permeation (Inagi et al 1981; Fang et al 2003). The results indicated that morphine and MPR permeation was influenced by proteins in the stratum corneum to a more-significant level than that of MEN. Two routes are involved in the transdermal delivery of drugs through the stratum corneum: permeation through corneocytes (i.e. a transcellular pathway) and permeation through the lipid bilayers surrounding the corneocytes (i.e. an intercellular pathway) (Lee et al 1997). The hydrophilic pathway through corneocytes may play an important role in the delivery of morphine and MPR via the stratum corneum. The intercellular lipid bilayers are still the predominant pathway for the extremely lipophilic MEN.

α-Terpineol and oleic acid have been used as permeation enhancers. Terpenes have been shown to act at the lipid polar heads of ceramides (Jain et al 2002; Panchagnula et al 2004), while fatty acids act at the lipidic tail portion of intercellular lipid bilayers (Gwak & Chun 2002; Jain & Panchagnula 2003). Morphine permeation showed the highest enhancement among three permeants when the skin was pretreated with both enhancers (Table 4). This suggested that the lipid bilayers were the main barrier blocking the transit of morphine. Intracellular or follicular routes may be the pathways for morphine delivery. The trend of decreasing permeation enhancement following the increase in drug lipophilicity suggests a more important role of the lipid bilayer in the permeation of lipophilic drugs. The slightly higher enhancement by α-terpineol than by oleic acid indicated that the diffusion along the lipid portion was more significant than that along the hydrogen bonds between ceramides.

Conclusions

The alkyl ester prodrugs of morphine, MPR and MEN, were successfully synthesized and the structures were verified in this study. The in-vitro transdermal delivery of morphine and its prodrugs was evaluated. The higher flux observed for the prodrugs, especially MEN, in the aqueous solution supported their superiority over morphine for transdermal delivery. The good skin permeation observed with these prodrugs could most likely be attributed to their high lipophilicity and moderate aqueous solubility. On the other hand, prodrugs in sesame oil showed lower permeation values compared with morphine. This indicated that the selection of a proper vehicle was necessary for the effective transdermal delivery of morphine and its prodrugs. The lipid bilayer lamellae within the stratum corneum were the predominant pathways for the ester prodrugs. The degradation rate of MEN by skin enzymes was higher than that of MPR. However, an opposite result was observed in the in-vitro transdermal delivery experiment. The more complicated condition of intact skin compared with the skin homogenate may have explained this discrepancy. The ester prodrugs of morphine showed the main requirements for transdermal prodrugs, including chemical stability, enzymatic lability and increased skin permeation. MPR and MEN appeared to be suitable candidates for morphine prodrug design.

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