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Utilization of prodrugs to enhance the transdermal absorption of morphine

Jørn Drustrup [1], Ann Fullerton [2], Lona Christrup [2] and Hans Bundgaard [1]

The Royal Danish School of Pharmacy, Departments of [1] Pharmaceutical Chemistry and [2] Pharmaceutics, Copenhagen (Denmark)

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Summary

The feasibility of providing transdermal delivery of morphine was examined using the prodrug approach. Various alkyl esters formed at the 3- and/or 6-hydroxy group in morphine were prepared and their physico-chemical and skin penetration properties studied as well as their hydrolysis kinetics. The esters showed generally a higher water and lipid solubility than morphine and were also much more lipophilic than the parent drug in terms of octanol-buffer partition coefficients. Diffusion experiments in vitro using human skin samples showed that whereas morphine did not penetrate the skin to any measurable extent whether applied in the form of saturated solutions in water at pH 7.0 or in isopropyl myristate, the ester prodrugs showed a high penetrating capacity under the same conditions. Steady-state fluxes up to 35 μg morphine/cm^2 per h were observed. For some esters essentially all of the amounts penetrated were present in the receptor phase as morphine. The study demonstrates the feasibility of achieving transdermal delivery of morphine based on the ready conversion and the favourable skin penetration properties of morphine esters which in turn are attributed to their combination of adequate water solubility and lipophilicity.

Introduction

During recent years attention has been paid to the development of transdermal delivery systems of narcotic analgesics as an alternative to the parenteral or oral means of delivering these agents (Schulte et al., 1980; Sebel et al., 1987; Duthie et al., 1988; Ritschel and Barkhaus, 1988; Aungst et al., 1989; Mahjour et al., 1989; Rieg-Falson et al., 1989; Ritschel et al., 1989; Roy and Flynn, 1989a; Sugibayashi et al., 1989). As pointed out by Roy and Flynn (1989b) and Ritschel et al. (1989), transdermal delivery may offer a means of mitigating some of the drawbacks associated with the parenteral or oral route of administration such as side effects due to high plasma peaks, too frequent dosing, and unpredictable and incomplete oral bioavailability due to extensive first-pass metabolism. A prerequisite for the development of a transdermal delivery system of morphine and other opioids is, however, that the drugs are capable of penetrating the skin at a sufficiently high rate and are not metabolized during the percutaneous absorption. Due to their favourable physico-chemical properties (water and lipid solubilities) and relatively high potencies fentanyl, sufentanil and meperidine may be successfully transdermally de-

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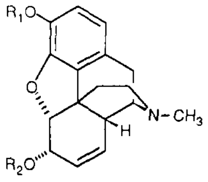
Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

livered (Sebel et al., 1987; Duthie et al., 1988; Ritschel et al., 1989; Roy and Flynn, 1989b). Morphine (**I**) which still remains the analgesic drug of choice for the treatment of severe chronic pain shows, however, a very limited skin permeability which may make it unsuited for transdermal delivery. Thus, the steady-state flux of morphine through human skin in vitro has been reported to be only 6 ng/cm^2 per h when applied in the form of a saturated aqueous solution (pH 7.4) (Roy and Flynn, 1989b). The poor skin penetration properties of morphine was mainly ascribed to its poor lipophilicity. The pK_a value of the amino function in morphine is 8.1 at 35°C (Roy and Flynn, 1989a) and its log P is only -0.15 where P is the partition coefficient between octanol and aqueous buffer of pH 7.4 at 35°C (Roy and Flynn, 1988).

The objective of the present work was to examine the prodrug approach to enhance the skin penetration of morphine in order to make transdermal delivery of the drug feasible. In this approach, which in the past few years has been increasingly used to optimize the dermal delivery of a variety of drugs (for reviews, see Bucks, 1984; Hadgraft, 1985; Sloan, 1989), the physico-chemical properties of the drug are changed in order to optimize the biphasic solubility and hence increase the diffusion rate through the skin, primarily the stratum corneum. To be a successful approach the prodrugs should further be cleaved quantitatively to the parent drug during or following skin penetration, and the pro-moiety released should be innocent.

Bioreversible derivatization of morphine (**I**) can readily be achieved by esterification of its 3-phenolic or 6-alcoholic group, or both, and in fact several esters of morphine have long been known including the diacetyl ester (diamorphine or heroin) (Beckett and Wright, 1875; Merck, 1899; Emde, 1930; Mannich and Siewert, 1939; Welsh, 1954; Zirm and Pongratz, 1959; May and Jacobsen, 1977; Owen et al., 1984; Sy et al., 1986; Broekkamp et al., 1988). The 3,6-dinicotinoyl ester (nicomorphine) is the only ester currently used clinically. However, the dermal delivery characteristics have not been examined for any ester of morphine. In the present study, various aliphatic esters of morphine (**II–IX**) were prepared and characterized with respect to solubility, lipophilicity, chemical stability and ability to permeate human skin as evaluated by diffusion experiments in vitro.



	R_1	R_2
I	H	H
II	CH_3CH_2CO-	CH_3CH_2CO-
III	CH_3CH_2CO-	H
IV	H	CH_3CH_2CO-
V	$(CH_3)_2CHCO$-	$(CH_3)_2CHCO$-
VI	$(CH_3)_2CHCO$-	H
VII	H	$(CH_3)_2CHCO$-
VIII	$CH_3(CH_2)_4CO$-	$CH_3(CH_2)_4CO$-
IX	$CH_3(CH_2)_4CO$-	H

Formule 1

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was performed with a system consisting of a Shimadzu pump Model LC-6A, a Shimadzu variable-wavelength UV detector Model SPD-6A and a 20-μl loop Rheodyne injection valve. A deactivated reversed-phase Supelcosil LC-8-DB column (33 × 4.6 mm) (3-μm particles) equipped with a Supelcoguard pre-column (purchased from Supelco Inc., U.S.A.) was used. Readings of pH were carried out on a Radiometer PHM 83 Autocal pH-meter at the temperature of study. Melting points were taken in capillary tubes.

Preparation of morphine esters

Morphine hydrochloride was obtained from Mecobenzon A/S, Copenhagen. The free base form was prepared by adding 200 ml of a saturated aqueous solution of sodium bicarbonate to a hot (50–60°C) solution of 10 g of morphine hydrochloride in 100 ml of water. The precipitate formed upon standing at 4°C for 4 h was filtered off, washed with water and recrystallized from methanol, m.p. 254–255°C (rep. m.p. 254–256°C (Muhtadi, 1988)).

The 3,6-diesters of morphine (**II**, **V** and **VIII**) were prepared by reacting morphine with an excess of the corresponding acid anhydride according to the method described by Owen et al. (1984). A typical procedure was as follows: A mixture of morphine (1.25 g, 4.4 mmol) and acid anhydride (25 mmol) was heated at 100°C for 4 h, cooled to room temperature and treated with water (20 ml). After 1 h, ether (25 ml) was added to the clear solution followed by a 10% sodium hydroxide solution to give a pH of 9–10. The ether layer was separated, washed with water, dried over anhydrous sodium sulphate and evaporated under reduced pressure. Compounds **II** and **V** were recrystallized from ethanol-water; compound **VIII** was an oil.

The 3-esters of morphine **III**, **VI** and **IX** were prepared by reacting morphine with the corresponding anhydride in weakly basic aqueous solutions using a previously described procedure (Welsh, 1954; Sy et al., 1986). The 6-propionyl ester of morphine (**IV**) was prepared as described by Sy et al. (1986) whereas the 6-isobutyryl ester (**VII**) was prepared in situ by alkaline hydrolysis of the corresponding diester (**V**).

Determination of solubilities and partition coefficients

The solubilities of morphine and the morphine esters were determined in triplicate in a phosphate buffer solution of pH 7.0 and in isopropyl myristate at 21 ± 1°C by placing excess amounts of the compounds in 2–5 ml of the solvent. The mixtures were placed in an ultrasonic bath for 10 min and then rotated on a mechanical spindle for 24 h and filtered. After rotation for 1 h the pH of the phosphate buffer mixtures was adjusted to 7.0. An aliquot of the filtrates was diluted with water or acetonitrile and analyzed by the HPLC methods as described below. In the case of morphine in isopropyl myristate the saturated solution was extracted with 0.1 M hydrochloric acid before analysis.

The pH-solubility profile of 3,6-dipropionylmorphine (**II**) was determined by the phase-solubility technique at 21°C. A 100 mg sample of **II** was dissolved in water with the aid of 1 M hydrochloric acid. The pH of the solution was 3.0. The solution was stirred at 21°C and 2 M sodium hydroxide was added dropwise. After each addition the mixture containing precipitated ester **II** was stirred for 1 h, the pH was recorded and an aliquot of 100 μl was withdrawn. Each aliquot was centrifuged and the clear supernatant analyzed by HPLC as described below upon appropriate dilution with water. It was shown that the solubility did not change significantly if the equilibration of solution was continued for more than 1 h after each addition of base.

The apparent partition coefficients (distribution coefficients) of morphine and various esters were determined at 21°C in an octanol-0.02 M phosphate buffer (pH 7.4) system. The concentration of the compounds in the aqueous phase before and after partitioning was determined by HPLC analysis, and the partition coefficients calculated as described before (Bundgaard et al., 1986).

The lipophilicity of the derivatives was also evaluated by means of reversed-phase chromatography as previously described (Bundgaard et al., 1986). In this method the capacity factor (k') of a solute is taken as a measure for the relative lipophilicity:

$$k' = (t_R - t_0)/t_0 \qquad (1)$$

where t_R is the retention time of the solute and t_0 is the elution time of the solvent. The solvent used was acetonitrile-methanol-0.01 M phosphate buffer pH 6.50 (3 : 1 : 6 v/v).

Hydrolysis of morphine esters

The hydrolysis of the esters **II**–**X** was studied in aqueous buffer solutions at constant temperature (±0.2°C). The buffers used were hydrochloric acid, acetate, phosphate, borate and carbonate buffers; the total buffer concentration was 0.02–0.1 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The reactions were initiated by adding 25–50 μl of a stock solution of the esters in acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being 5×10^{-6}–10^{-4} M. The solutions were kept in a water bath at constant temperature and at appropriate intervals samples were taken and immediately chromatographed. Pseudo-first-order rate constants for the degradation of the esters were determined from the slopes of linear plots of the logarithm of residual ester against time.

The hydrolysis of the esters were also studied in human serum. The compounds were incubated at 37°C in human serum diluted to 80% with 0.05 M phosphate buffer of pH 7.40. The initial concentration of the esters was 5×10^{-5} M. At appropriate intervals, samples of 250 μl of the serum reaction solutions were withdrawn and added to 500 μl of acetonitrile in order to deproteinize the serum. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Permeability-metabolism studies using excised human skin

Whole abdominal human skin obtained under autopsy from two donors was used. The skin was stored at −18°C and was allowed to thaw gradually at room temperature before use. All subcutaneous fat was removed and the skin cut into pieces. The excised skin was mounted in open diffusion cells of the same type as those used by Franz (1975); they have an available diffusion area of 0.70 cm^2. The dermal side of the skin was exposed to the receptor medium (5.2 ml of 0.05 M isotonic phosphate buffer of pH 7.2) which was stirred magnetically and kept at a constant temperature of 37°C with a circulating water bath. The skin in the assembled diffusion cells was initially screened for barrier integrity by applying 195 μl [^{3}H]water to the surface of the skin for 18 h. The 18 h permeation was compared to the value for intact skin.

The compounds studied were applied as solutions or suspensions (200 μl). The suspensions were allowed to stir for 24 h prior to application to the skin surface. At appropriate intervals samples of 1 ml were removed from the receptor phase and replaced with fresh buffer. The samples were stored at −20°C until they were analyzed for morphine, di- and/or monoester content by HPLC as described below. The permeation studies of each compound were done in tri- or quadruplicate.

HPLC analysis of morphine and its esters

Reversed-phase HPLC procedures were used for the quantitative determination of morphine and its esters. A deactivated Supelcosil column was eluted with a mobile phase consisting of a mixture of acetonitrile (15–70% v/v), methanol (10% v/v) and 0.01 M phosphate buffer solution of pH 6.5. The concentration of acetonitrile was adjusted for each compound to give a suitable retention time (3–10 min) of the compound. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 215 or 280 nm. It was assured that in each case adequate separation of the ester from morphine and monoesters (in the case of the diesters) was achieved. Quantitation of the compounds was done from measurements of the peak heights in relation to those of standards chromatographed under the same conditions.

Results and Discussion

Chemical stability of morphine esters

The kinetics of hydrolytic breakdown of the various morphine esters (Fig. 1) was studied in aqueous solution at 37 and 60°C over a wide pH range. Under the experimental conditions used the degradation of the esters displayed strict first-order kinetics for several half-lives. Typical first-order plots for the degradation are shown in Fig. 1.

The rate of hydrolysis of the esters were subject to general acid-base catalysis by most buffers used to maintain constant pH. The buffer-independent pseudo-first-order rate constants (k) were determined by extrapolation of linear plots of the observed pseudo-first-order rate constants (k_{obs}) vs total buffer concentration to zero concentration.

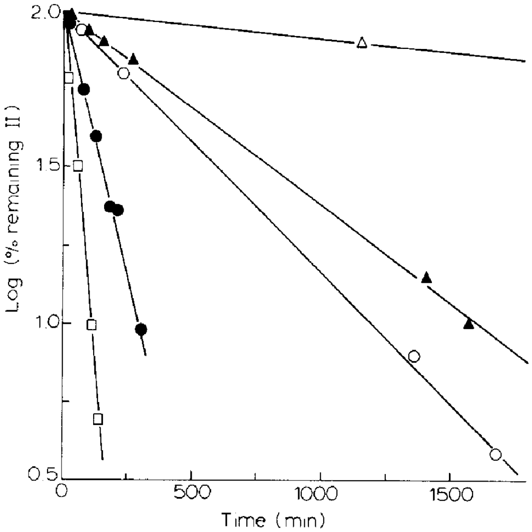


Fig. 1. First-order plots for the overall degradation of 3,6-dipropionylmorphine (**II**) in aqueous solution ($\mu = 0.5$). (△) 0.05 M phosphate buffer pH 7.40 (37°C); (▲) 0.02 M borate buffer pH 8.40 (37°C); (○) 0.05 M phosphate buffer pH 7.40 (37°C); (●) 0.1 M hydrochloric acid pH 1.10 (60°C); (□) 0.1 M borate buffer pH 8.30 (60°C).

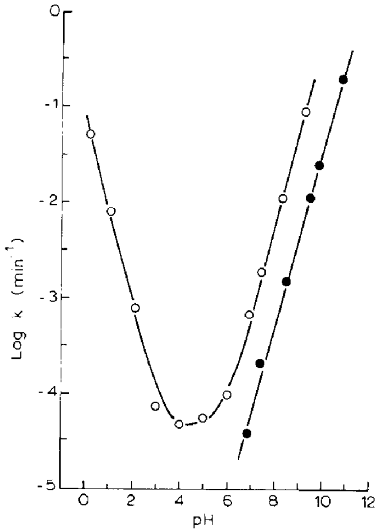


Fig. 2. The pH-rate profiles for the hydrolysis of 3,6-dipropionylmorphine (**II**) in aqueous solution ($\mu = 0.5$) at 37 (●) and 60°C (○).

The effect of pH upon the hydrolysis of the dipropionyl ester **II** was examined in detail over the pH range 0.4–9.4 whereas the other esters only were studied in acidic and basic solutions. As seen from Fig. 2 the pH-rate profile for **II** is U-shaped, indicating the occurrence of specific acid- and base-catalyzed as well as spontaneous or water-catalyzed reactions according to the following rate expression:

$$k = K_0 + k_H a_H + k_{OH} a_{OH} \qquad (2)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. The latter was calculated from the measured pH at 37 or 60°C according to the following equations (Harned and Hamer, 1933):

$$\log a_{OH} = \mathrm{pH} - 13.62\ (37°\mathrm{C}) \qquad (3)$$

$$\log a_{OH} = \mathrm{pH} - 13.02\ (60°\mathrm{C}) \qquad (4)$$

Values of the second-order rate constants for the specific acid (k_H) and specific base (k_{OH}) catalyzed hydrolysis were determined from the straight line portions of the pH-rate profiles at low and high pH values, respectively, whereas the value of the first-order rate constant for spontaneous hydrolysis (k_0) of **II** was obtained on the basis of Eqn 2. The values of the rate constants derived for ester **II** and the other esters are listed in Table 1. Maximal stability of the dipropionyl ester **II** occurs at pH 4 which is similar to that reported for diacetylmorphine (Beaumont, 1982).

TABLE 1

Rate data for the hydrolysis of various esters of morphine in aqueous solution ($\mu = 0.5$) at 60°C

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})
II	0.085	4.1×10^{-5}	625 (200) [a]
III	0.090		800
IV	0.031		95
V	0.025		150 (45) [a]
VI	0.032		330
VIII	0.040		– [b]
IX	0.045		300 (74) [a]

[a] Values in paranthesis are at 37°C.

[b] Due to solubility reasons the hydrolysis of **VIII** was not studied in basic solutions.

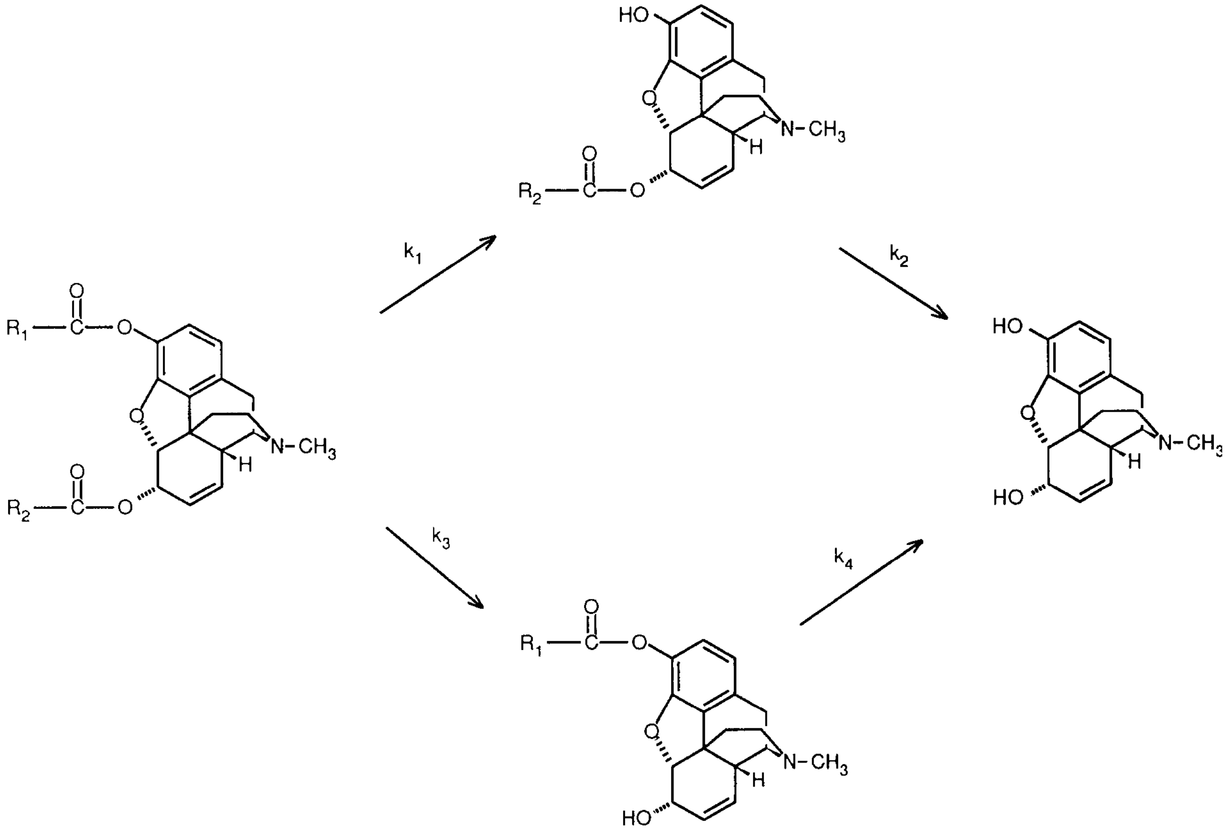
The morphine esters are weak bases due to the tertiary amino group. As described later the pK_a value of the diester **II** is 8.3 at 21°C and $\mu = 0.5$ as determined from solubility data Since the pH-rate profiles for the esters do not show a significant curvature at pH values near to the pK_a value, the free base and protonated forms exhibit the same reactivity toward hydroxide ion-catalyzed hydrolysis.

Whereas the 3- and 6-monoesters of morphine degraded directly to yield morphine in quantitative amounts, the diesters were found to degrade into morphine via the intermediate formation of the corresponding 6-monoester (Scheme 1 with $k_1 \gg k_3$). The other possible degradation route is initial hydrolysis of the ester function at C-6 yielding the 3-monoester, but this pathway was found to make no significant contribution (< 1%) to the overall degradation as demonstrated by HPLC analysis of the reaction solutions. This behaviour is in accordance with the better leavability of the 3-phenolate group relative to the 6-alkoxide group (cf. the k_{OH} values for **III** and **IV** in Table 1). Similar findings have been observed in the basic hydrolysis of diacetyl and dinicotinoyl esters of morphine (Nakamura et al., 1975; Lindner and Semmelrock, 1981; Lobbezoo et al., 1982).

The time courses of the dipropionyl ester **II**, the 6-propionyl ester **III** and morphine (**I**) during hydrolysis of compound **II** in a 0.05 M borate buffer solution of pH 9.3 are shown in Fig. 3. The curves drawn were calculated from the following expressions:

$$\%\mathbf{II}_t = 100 \times e^{-k_1 t} \tag{5}$$

$$\%\mathbf{III}_t = 100\left(\frac{k_1}{k_1 - k_2}\right)\left(e^{-k_2 t} - e^{-k_1 t}\right) \tag{6}$$



Scheme 1

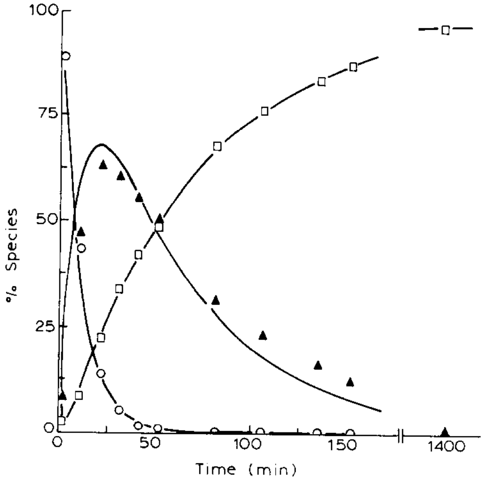


Fig. 3. Time courses for the degradation of 3,6-dipropionylmorphine (**II**) in 0.05 M borate buffer solution pH 9.30 at 60°C. (○) **II**; (▲) **IV**; (□) **I**.

$$\%\mathrm{I}_t = 100\left(1 - \left(\frac{k_2 e^{-k_1 t} - k_1 e^{-k_2 t}}{k_2 - k_1}\right)\right) \qquad (7)$$

where k_1 and k_2 are pseudo-first-order rate constants for the reactions depicted in Scheme 1. The following values were used in the calculations: $k_1 = 0.098$ min^{-1} and $k_2 = 0.019$ min^{-1}. This value of k_1 was identical within ±5% to the k value for the total disappearance of the diester. Combined with the failure of detecting significant amounts (< 1%) of the 3-monoester during the degradation of the diester **II** in neutral and alkaline solutions, this demonstrates the validity of Scheme 1 with $k_1 \gg k_3$ as describing the overall hydrolysis kinetics.

Considering the values of k_{OH} in Table 1 it is interesting to note that the diesters **II** and **V** are more stable than the corresponding 3-monoesters (**III** and **VI**). This may possibly imply that the 6-OH group exhibits a catalytic effect albeit slight on the hydrolysis of the 3-esters. In acidic solutions, on the other hand, the 3-monoesters and 3,6-diesters possess almost the same stability.

Hydrolysis in human serum

As an indication of the susceptibility of the morphine esters to undergo conversion to morphine in vivo their stability was examined in the presence of human serum at 37°C. The kinetic data obtained are shown in Table 2. Under the experimental conditions used the 3-monoesters and the 3,6-diesters degraded according to first-order kinetics (Fig. 4), the latter compounds being hydrolyzed quantitatively to the corresponding 6-esters. As seen from the data in Table 2 the ester group at C-3 is rapidly hydrolyzed both in 3-monoesters and in the diesters whereas the ester function at C-6 is quite resistant to attack by serum enzymes (less than 5% cleavage after 24 h). Similar findings have been reported for the acetyl

TABLE 2

Half-lives of hydrolysis of various morphine esters in 0.05 M phosphate buffer solutions and in human serum

Compound	Half-life (min)		
	pH 7.4 (60 °C)	pH 7.4 (37 °C)	80% human serum (37°C)
II	360	3250	4.2
III	230		< 0.1
IV	2100		> 1440
V	1060		300
VI	325	2950	0.6
VII			> 1440
VIII			< 0.2
IX	960	~ 9000	< 0.1

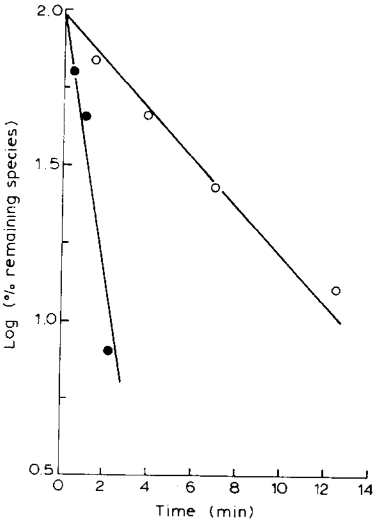


Fig. 4. First-order plots for the overall degradation of 3,6-dipropionylmorphine (**II**) (○) and 3-isobutyrylmorphine (**VI**) (●) in 80% human serum solution (pH 7.4) at 37°C.

and nicotinoyl esters of morphine (Wright, 1941; Nakamura et al., 1975; Lockridge et al., 1980; Lindner and Raab, 1981; Lobbezoo et al., 1982; van Rooy et al., 1984; Oldendorf and Stoller, 1989) as well as for the dipropionyl and dibutyryl esters (Owen and Nakatsu, 1984).

Solubility and lipophilicity

The solubilities of morphine and some esters in water at pH 7.0 and in isopropyl myristate (IPM) at 21°C are shown in Table 3. Except for the highly lipophilic dihexanoyl ester **VIII**, the esters show an increase in both water and lipid solubility relative to morphine. This higher biphasic solubility may be most favourable for skin penetration and is reflected in the decreased melting points of the esters as compared to morphine. The high melting point of morphine has been attributed to its great ability to self-associate within the crystalline state (Roy and Flynn, 1988). Apparently, this behaviour can be strongly depressed by esterification of the molecule.

The aqueous solubility of the 3,6-dipropionyl ester of morphine (**II**) was determined at 21°C as a function of pH. The pH-solubility profile obtained is shown in Fig. 5. It can be accounted for by Eqn 8:

$$S_T = [B]_s(a_H + K_a)/K_a \qquad (8)$$

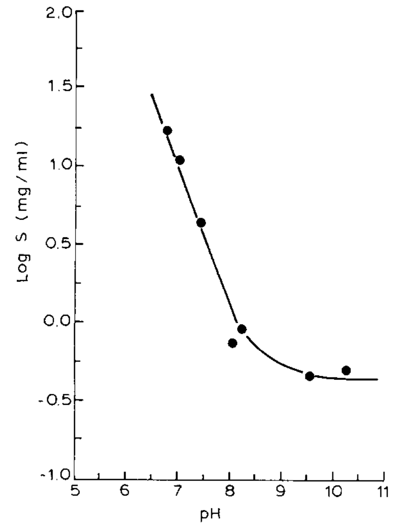


Fig. 5. The pH-solubility profile for 3,6-dipropionylmorphine (**II**) at 21°C. The points are experimental while the curve is calculated from Eqn 8.

where S_T is the total solubility of the compound, $[B]_s$ is the intrinsic solubility of the free base, a_H is the hydrogen ion activity and K_a is the ionization constant of protonated **II**. In Fig. 5 the curve drawn is constructed from Eqn 8 and the following values of $[B]_s$ and K_a: $[B]_s = 0.45$ mg ml^{-1} and $K_a = 10^{-8.3}$.

As seen from Table 3 all the esters are more lipophilic than the parent drug in terms of partition coefficients between octanol and a phosphate buffer of pH 7.4. The figure for morphine is in good agreement with that (−0.15) reported by Roy and Flynn (1988) at 37°C whereas the figures of the esters mutually correspond well to those predicted from the hydrophobic π substituent constants (Hansch and Leo, 1979). The lipophilicity of the derivatives was also evaluated by means of HPLC capacity factors (k'). Using the solvent system described in Materials and Methods the following linear relationship was found between $\log k'$ and $\log P$ for compounds **I**–**III**, **V**, **VI** and **IX**:

$$\log P = 6.65 \log k' - 0.84 \ (n = 6;\ r = 0.999) \qquad (9)$$

TABLE 3

Melting points, solubilities and partition coefficients of morphine and various 3-mono- and 3,6-diesters of morphine

Compound	m.p. (°C)	Solubility (mg/ml) (21°C)		log P [a]
		pH 7.0 buffer	IPM	
I	254–255	1.8	0.023	−0.06
II	106–107	3.6	41	1.66
III	oil	21	79	0.66
IV	176–177			0.52 [b]
V	96– 97	0.60	79	2.60
VI	oil			1.15
VIII	oil	0.02	> 200	> 4 [c]
IX	oil	2.6	>150	2.04

[a] P is the partition coefficient between octanol and a phosphate buffer of pH 7.4 (21°C).
[b] Predicted value from Eqn 9.
[c] Predicted log P = 4.66 on basis of n substituent constants.

Skin permeation

Excised human skin was used to examine the percutaneous absorption of morphine and the morphine esters **II**, **VIII** and **IX**. Each compound was studied in both a polar (i.e., an aqueous phosphate buffer of pH 7.0) and an apolar (i.e., isopropyl myristate) vehicle. Suspensions of the compounds in the vehicles except for **VIII** and **IX** in IPM were applied in order to keep a constant driving force for diffusion and to provide the maximum flux attainable which is the property to be optimized in transdermal drug delivery.

In the case of morphine no measurable amounts of drug could be detected in the receptor phase during diffusion experiments lasting up to 200 h. The failure of morphine to penetrate human skin from the vehicles applied in significant amounts is in accordance with the results obtained by Roy and Flynn (1989b). These authors reported a steady-state flux of 0.006 $\mu g/cm^2$ per h for the permeation of morphine through human skin from a saturated solution of the drug in a pH 7.4 buffer.

In contrast, the morphine esters **I, VIII** and **IX** readily penetrated human skin. The results obtained with these prodrugs are shown in Fig. 6 in which the cumulative amounts (in μg morphine base) of morphine or ester measured in the receptor phase divided by the surface area of the diffusion cell are plotted against the time of sampling. The steady-state fluxes were obtained from the slopes of the linear portions of these plots. The permeability coefficients (K_P) for the steady-state delivery were obtained by dividing the steady-state fluxes by the solubilities of the compounds in the vehicle applied. The values obtained for these parameters are given in Table 4.

In the case of the hexanoyl esters **VIII** and **IX**, only morphine was found in the receptor phase whereas for the 3,6-dipropionyl ester **II** approx. 50% of the amounts penetrated were present in the receptor phase as morphine and 50% as the corresponding 6-monoester (**IV**). The flux and K_P values given in Table 4 for compound **II** were calculated in terms of total morphine equivalents. The ready ability of the 3-monoester and the diesters to be hydrolyzed during the transport through the skin is not surprising in view of their facile enzymatic hydrolysis observed in human serum as

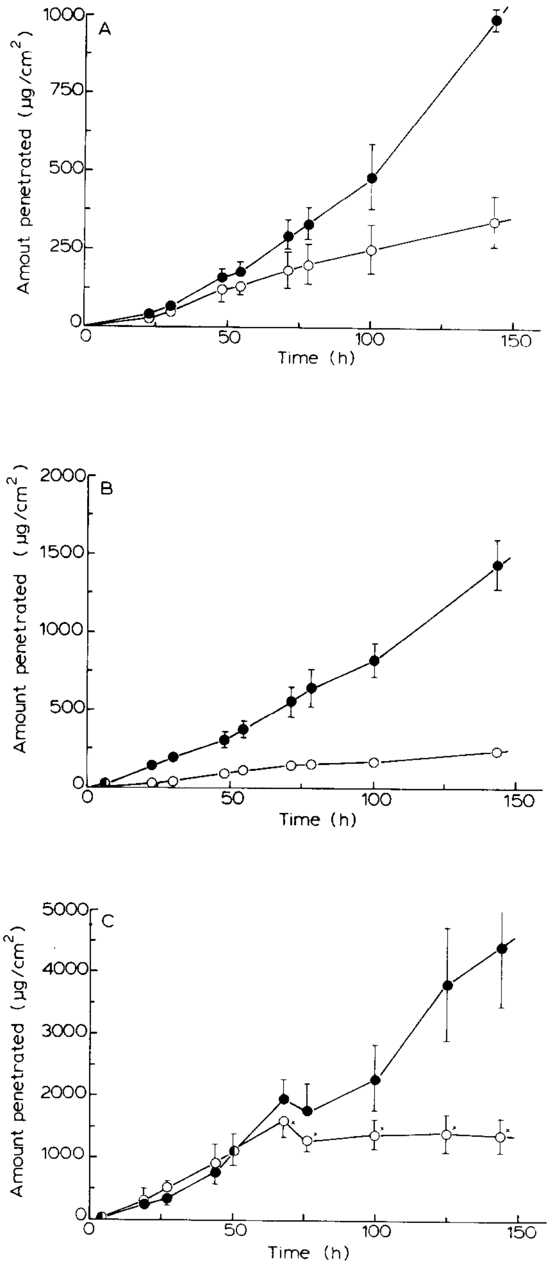


Fig. 6. Permeability of morphine esters through human skin as amount of morphine appearing in the receptor phase as function of time from suspensions or solutions of compound **II** (A), compound **VIII** (B) and compound **IX** (C) in 0.05 M phosphate buffer of pH 7.0 (○) and isopropyl myristate (●). Compound **II** was applied as suspensions in both buffer and IPM, compounds **VIII** and **IX** as suspensions in buffer and as solutions (200 mg ml^{-1} (**VIII**), 125 mg ml^{-1} (**IX**)) in IPM. In panel C, ☆ indicates that all the morphine ester applied has penetrated the skin. Error bars are ± S.E.

TABLE 4

Fluxes and permeability coefficients (Kp) for steady-state phase of delivery of morphine through human skin from isopropyl myristate (IPM) and an aqueous buffer of pH 7.0

Compound	Flux ($\mu g/cm^2$ per h) ($\pm$S.E.)		K_p (cm/h)	
	IPM	Buffer	IPM	Buffer
I	< 0.01	< 0.01	$< 4.3 \times 10^{-4}$	$< 5.6 \times 10^{-6}$
II [a]	8.7 ± 0.4	2.5 ± 0.5	3.0×10^{-4}	1.0×10^{-3}
VIII	11.7 ± 1.2 [b]	1.7 ± 0.2	$> 1.6 \times 10^{-4}$	0.14
IX	35.6 ± 12.0 [c]	25.3 ± 4.2	$> 2.4 \times 10^{-4}$	1.3×10^{-2}

[a] Approx. 50% of the amounts penetrated were present in the receptor phase as morphine and 50% as the 6-monoester. The flux values given were calculated in terms of total morphine equivalents.

[b] The IPM solution applied was not saturated. It contained the compound at a concentration of 200 mg ml^{-1}.

[c] The IPM solution applied was not saturated. It contained the compound at a concentration of 125 mg ml^{-1}.

reported above. However, it is interesting to note the quite efficient enzymatic hydrolysis of the 6-monoesters formed upon the initial hydrolysis of the diesters. Thus, whereas the serum-catalyzed hydrolysis of these esters is very slow, the skin enzyme-mediated hydrolysis during the diffusion experiments is appreciable, notably for the hexanoyl ester which was completely converted to morphine during the diffusion.

The results obtained from the diffusion experiments show that it is possible to improve very markedly the skin penetration of morphine via prodrugs. Thus, the 3-hexanoyl ester afforded a more than 2000-fold higher flux relative to morphine itself when delivered from an aqueous buffer vehicle, and an even greater enhancement was achieved when isopropyl myristate was used as a vehicle. The increased solubility of the esters in the vehicles combined with expected concomitant increase in solubility in the skin is most certainly responsible for the higher fluxes, since the ability of the esters to partition into the skin is part of the driving force for diffusion. Comparing the fluxes and solubilities of the compounds studied further show that the results obtained are in accordance with the general trend observed for various prodrugs designed to enhance skin penetration that the most effective derivatives are the ones which combine an increased lipid and an increased water solubility over the parent drug (Sloan, 1989).

The feasibility of obtaining transdermal delivery of morphine via the prodrug approach can be assessed by comparing the daily doses of morphine used via parenteral administration with those obtainable by transdermal delivery. If the area of the patch for transdermal delivery is 25 cm^2 and if a flux of 25 μg/h per cm^2 is used, it would be possible to deliver 625 μg morphine/h or 15 mg over 24 h. This amount is higher than that usually given (10 mg) during 24 h.

Conclusions

In conclusion, this study shows that the transport of morphine through human skin can be markedly enhanced by using more lipophilic prodrugs. The fluxes observed for the morphine esters, in particular those for the 3-hexanoyl ester, from both a polar and an apolar vehicle, suggest the feasibility of developing a transdermal delivery system for morphine based on the good skin penetration properties and ready bioconversion of the ester prodrug.

It should be added that the fluxes observed can most likely be further enhanced by various formulation techniques including the use of penetration enhancers. It should also be noted that the fluxes observed for the esters **VIII** and **IX** from the isopropyl myristate vehicle do not represent maximally obtainable values since the esters were not applied in the form of saturated solutions. Finally, it is recognized that the esters studied do not necessarily represent the optimum ones in terms of rate of transport through the skin.

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