Transdermal Prodrug Concepts: Permeation of Buprenorphine and Its Alkyl Esters through Hairless Mouse Skin and Influence of Vehicles

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In vitro skin permeation of buprenorphine (BUP) and three of its alkyl ester prodrugs was evaluated using hairless mouse skin. The three esters selected were the acetyl ester (Ac-BUP), butyl ester (Bu-BUP), and isobutyl ester (Isb-BUP). These drugs were applied on the skin as saturated slurries in three vehicles commonly used to formulate agents for transdermal purposes: propylene glycol, polyethylene glycol 400 (PEG 400), and light mineral oil. Unique solubilities were found for each drug on each vehicle. Fluxes through hairless mouse skin were evaluated for each combination of drug and vehicle using Franz diffusion cells. From PEG 400 formulations, the skin fluxes of BUP, Ac-BUP, Bu-BUP, and Isb-BUP were 0.47 ± 0.08 , 1.64 ± 0.31 , 0.33 ± 0.05 , $0.75 \pm 0.20 \mu\text{g}/\text{cm}^2/\text{h}$, respectively. Thus, among the three potential prodrugs chosen, only Ac-BUP showed significantly higher skin flux than BUP. There were no inter-vehicle differences in the fluxes from saturated slurries between the vehicles. Moreover, all the esters were detected substantially in the form of regenerated parent drug (BUP) in the receptor compartment. Indeed, only Ac-BUP exited the skin in a measurably intact form, but the fraction escaping metabolism in transit was small (approximately 2%). However, based on drug dispositions in the skin, the regeneration of buprenorphine seems to depend on the alkyl chain length of the ester moiety. The molar percentages of regenerated parent drug in whole drug collected from the skin following the permeation experiments were: Ac-BUP, 9.2%; Bu-BUP, 40.7%; Isb-BUP, 9.6%, respectively. Thus, only Ac-BUP appears promising as a prodrug of buprenorphine, because it is not overly hydrophilic for skin permeation and is also highly metabolized to the parent compound while in the skin.

Key words buprenorphine; alkyl ester prodrug; transdermal drug delivery; hairless mouse skin; vehicle effect

Buprenorphine is a synthetic agonist-antagonist opiate whose potency is 100 times greater than morphine's.²⁾ It is clinically used as an analgesic, but it can be also used as a safe substitute for heroin (street opiate) in the treatment of narcotic addiction.³⁾ Buprenorphine is presently mainly administrated parenterally for pain and sublingually in drug abuse treatment. Either way, doses of the drug are boluses which give rise to immediately high levels of the drug in the blood which taper off gradually between doses. The transdermal method of administration avoids the unfavorable pharmacokinetic and toxicological consequences of such pulsatile dosing. This method of administration would be ideal in drug abuse treatment if sufficient drug could be delivered from a practically sized patch.

Buprenorphine, because of its high crystallinity, is not likely to be a good candidate for transdermal drug delivery by itself.⁴⁾ Utilization of hydrochloride salt, along with a permeation enhancer, has been offered as one solution to obtain sufficient skin permeation of buprenorphine for analgesic purposes.⁵⁾ A prodrug strategy is another possibility.⁶⁾ The addition of an alkyl chain to the drug molecule can produce a higher free energy form of a compound by interfering with intracrystalline association.⁷⁾ We have already shown that 3-alkyl ester formation gives more favorable physicochemical properties to the compound as required to improve its flux across a lipoidal membrane.⁴⁾

In this study, to determine the actual change in skin permeability through alkyl ester prodrug modification, the permeation of buprenorphine and its three typical alkyl esters across full thickness hairless mouse skin was evaluated. At the same time, with an eye to the design of a transdermal system for buprenorphine, delivery from

some prospective vehicles⁸⁾ was also investigated.

MATERIALS AND METHODS

Materials Buprenorphine base (BUP) was purchased from AKZO (Diosynth, The Netherlands). The alkyl esters: acetyl ester (Ac-BUP), butyl ester (Bu-BUP), and isobutyl ester (Isb-BUP), were synthesized as reported previously.⁴⁾ In brief, buprenorphine base was reacted with the respective aliphatic acid anhydride in the presence of dimethylaminopyridine. Purity (>98%) was assured through elemental analysis, ¹H-NMR spectroscopy, HPLC and by the sharpness of melting points. Polyethylene glycol 400 (PEG 400) was obtained from Sigma Chemicals (St. Louis, MO, U.S.A.). Light mineral oil (OIL) and propylene glycol (PG) were purchased from Fisher (Fair Lawn, NJ, U.S.A.). All other chemicals were reagent grade.

Preparation of Formulation The saturated drug slurries were prepared by equilibrating large excesses of the solutes with the respective solvents in temperature-controlled glass vessels. Each slurry was stirred vigorously for more than 24 h with a magnetic bar at 37 °C with jacketed circulating water.

Solubility Determination Each slurry was filtered through a warmed glass microfibre filter (GF/C Whatman, Maidstone, England), and an amount of the filtrate was appropriately diluted with acetonitrile and assayed by HPLC.

Skin Preparation and Permeation Experiment Franz diffusion cells (effective area: 0.785 cm², Crown Glass, NJ, U.S.A.) were used in the permeation study. Eight-and-one-half percent (w/w) PEG-400/ water was used as the receptor fluid. The receptor fluid was degassed before use

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by a vacuum filtration method. The receptor compartments of the diffusion cells were stirred at 600 rpm by a magnetic stirrer placed in the bottom of the cells. The temperature of the system was maintained at 37 °C using a circulation water bath (Lauda, Germany). The membranes used were full thickness skin sections taken from the abdominal and dorsal surfaces of the hairless mouse, SKH-hr-1 strain. Hairless mouse males 60 to 90 d of age were sacrificed by carbon dioxide suffocation. Rectangular sections of skin, several cm in each dimension, were excised from the animal. After removing all adhering fat from the skin's underside and trimming it into a square shape, the skin was mounted on a diffusion cell. The skin was equilibrated with receptor fluid for at least 1 h before applying one of the drug-containing formulations to the exposed surface of the skin. One-hundred-and-fifty μl of each formulation was used as the donor phase. The donor compartments of the diffusion cells were kept under an occluded condition covered with aluminum foil. The entire volume of receptor fluid was taken as the sample, with replacement with fresh receptor phase, and the sample was placed in a pre-weighed vial. This sampling routine was continued every 8 h for 48 h. After 48 h had elapsed and the last sample was taken, the skin was removed from the diffusion cell. Drug crystals remaining on the surface of the skin were removed by applying adhesive tape strips (Magic Tape, Scotch, St. Paul, MN, U.S.A.) two times, each with abrupt removal according to our usual tape stripping method. The skin was carefully trimmed with a surgical blade and then placed in a vial. Ten ml of acetonitrile were added to extract buprenorphine out of the tissue. The vials were gently agitated overnight to facilitate the extraction. The samples were then assayed by HPLC.

Assay Buprenorphine and its alkyl prodrugs were assayed by HPLC using UV detection. Chromatographic resolution was obtained on a C_8 reverse phase column (Brownlee RP8 Spheri-5, 5 μm , 220 $\times$ 4.6 mm, Applied Biosystems, CA, U.S.A.). The mobile phase was acetonitrile-methanol-pH 5.0 phosphate buffer (12:5:3), and the flow rate was 1.5 ml/min. The UV detector was set at 215 nm and the injection volume was 20 μl . The peak area was quantified with the aid of a Hewlett Packard integrator. A standard calibration curve was constructed using samples having known concentrations simply by plotting the peak area *versus* the concentration of drug.

Data Analysis Generally, the skin permeation phenomena of an ester prodrug involves a complicated process: chemical and/or enzymatic hydrolysis in the skin. In this study, in order to simply evaluate the change in skin permeation profile among several kinds of prodrugs, the cumulative amount of drug detected in each receptor fluid sample was calculated from the HPLC peaks and expressed as the total prodrug amount, *i.e.*, the sum of intact prodrug and regenerated parent drug, which required the following molecular weight adjustment:

$$\text{amount} = A + B \cdot (MW_{\text{pro}}/MW_{\text{parent}}) \quad (1)$$

where A is the amount of intact prodrug, B is that of regenerated parent drug, MW_{pro} is the molecular weight of prodrug, and MW_{parent} is that of the parent drug. The

steady state of skin flux was determined and expressed per unit of diffusion area in $\mu\text{g}/\text{cm}^2/\text{h}$ by a linear regression calculation from the slope of the linear portion of the cumulative amount permeated *versus* time plot.

The permeability coefficient P in cm/h was determined experimentally from:

$$J_s = P \cdot \Delta C \quad (2)$$

where J_s is the steady state skin flux and ΔC is the concentration differential across the skin in mg/ml. Since the receptor phase is maintained in a sink condition and the donor phase is kept saturated during the experiment, ΔC is actually very well approximated by the saturated concentration of the drug in question in its formulation.

RESULTS AND DISCUSSION

We have already reported that modification of buprenorphine's structure with an alkyl ester moiety accomplishes the physicochemical goal of reducing crystallinity and thereby raising solubilities in organic media.⁴⁾ These physicochemical changes are expected to improve the drug's flux across a lipoidal membrane. In this study, to determine the actual change in skin permeability through such modification, *in vitro* skin permeation was investigated using membranes prepared from hairless mouse skin. Three liquid vehicles which might be used in an eventual transdermal patch, propylene glycol (PG), polyethylene glycol 400 (PEG 400), and light mineral oil (OIL), were selected. The vehicles represent a wide range of polarities as reflected in their solubility parameters: PG: 15.0, PEG 400: 10.8, and OIL: 8.1 (cal/cm^3)^{1/2}.⁹⁾ The solubilities of the four drugs in the three kinds of

Compound	R
Buprenorphine (BUP)	—H
Acetyl-buprenorphine (Ac-BUP)	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—C—CH}_3 \end{array}$
Butyl-buprenorphine (Bu-BUP)	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—C—CH}_2\text{—CH}_2\text{—CH}_3 \end{array}$
Isobutyl-buprenorphine (Isb-BUP)	$\begin{array}{c} \text{O} \\ \parallel \\ \text{—C—CH} \begin{array}{l} \nearrow \text{CH}_3 \\ \searrow \text{CH}_3 \end{array} \end{array}$

Fig. 1. Chemical Structures of Buprenorphine and Its Alkyl Ester Prodrugs

Table 1. Solubility of Drugs in Three Kinds of Vehicles

Drug	Solubility at 37 °C (mg/ml)		
	Propylene glycol	PEG 400	Light mineral oil
BUP	7.4	23.3	0.68
Ac-BUP	4.8	19.4	19.8
Bu-BUP	0.98	3.7	10.0
Isb-BUP	4.1	14.9	45.1

Table 2. Physicochemical Properties of Buprenorphine and Its Alkyl Ester Prodrugs

Physicochemical properties	Drug			
	BUP	Ac-BUP	Bu-BUP	Isb-BUP ^{b)}
Activity of solid ^{a)} at 37 °C	0.0216	0.0766	0.0355	0.2140
log $P_{\text{oct/water}}$ ^{b)}	2.9	3.5	4.1	4.2

a) Calculated from ref. 4. b) Unpublished data.

vehicles are summarized in Table 1. In the polar vehicles, PG and PEG 400, the prodrugs exhibited lower solubilities than the parent drug. In nonpolar OIL, the prodrugs were more soluble than the parent drug. Isb-BUP was more soluble than Bu-BUP in all three vehicles. This latter observation is as expected, given the physicochemical properties; activity of solid, of these two close analogues (Table 2). It is realized that one cannot compare the activities of two compounds in conventional thermodynamics; however, in extrathermodynamic terms, the drug with higher activity will ordinarily exhibit higher solubility, especially in a non-aqueous media. This, of course, actually depends on how the crystallinity-modifying molecular addition affects the activity coefficient of the drug in the solvent of comparison. In the instance of buprenorphine and its three potential prodrugs, a phenolic hydroxyl group is capped with an ester linkage, removing a strong hydrogen bonding center and replacing it with a far less water interactive functionality. Moreover, the alkyl portion of the ester linkage in hydrophobic, which will further drive down solubility in water but will have an indeterminate influence on solubility in other solvents, does not structure itself at low energy surface. It can be seen that solubilities in OIL are benefited through the molecular modifications and reflect the gradual order of extrathermodynamic activities. This is as it should be, since the interaction between the drugs and OIL is confined strictly to dispersion forces. The physical measures of crystallinity, melting point and enthalpy of fusion indicate that Isb-BUP is far less crystalline than Bu-BUP, and this shows up not only in the OIL solubilities (Isb-BUP, 4.5 times higher than Bu-BUP) but in the solubilities in PG and PEG 400 as well (each also at a 4.5 times ratio). However, the esterification of buprenorphine does not result in higher solubilities of the potential prodrugs in either PG or PEG-400, despite favorable change in crystallinity. This simply means that one pays a price in solubility terms for the lost hydroxyl group, which would be interactive through H-bonding in both these solvents, and for the

Fig. 2. Representative Permeation Profiles of BUP (Δ), Ac-BUP ($\square$), Bu-BUP ($*$), and Isb-BUP ($\circ$) Through Hairless Mouse Skin from Saturated Drug Slurries in PEG 400 Vehicle

Vertical bars indicate the S.D. and each point is the mean value of three experiments. Lines calculated by the linear regression method are also shown.

Fig. 3. Skin Fluxes of BUP and Its Alkyl Ester Prodrugs Following Applications of Saturated Slurries in Three Kinds of Vehicles

PG (closed bar), PEG 400 (open bar), and OIL (hatched bar). Vertical bars indicate the S.D. of three experiments.

alkyl fractions. In saying this, it is wise to remember that neither PG nor PEG 400 is miscible with pure hydrocarbon solvents.

Representative plots of the cumulative amount of each drug (converted as the prodrug amount through Eq. 1) through hairless mouse skin from the PEG 400 vehicle as a function of time are shown in Fig. 2. Each data point on the graph is the mean of triplicate diffusion experiments. In the case of Ac-BUP, a small portion (approximately 2%) of the drug detected in the receptor fluid was the intact prodrug, while in the Bu-BUP and Isb-BUP cases, all drugs appeared as regenerated parent drug.

A saturated slurry was used for the skin permeation experiment to ensure that there was an unit thermodynamic activity and thus a common activity for drug in each formulation. The values of drug flux through the skin for each of the three vehicles are shown in Fig. 3. Percutaneous fluxes from saturated slurries of BUP and its three alkyl esters were relatively insensitive to the nature of the vehicles. In part, this outcome is in good accord with the percutaneous penetration theory which teaches us that the permeation rate should be directly proportional to the thermodynamic activity of

a drug in its vehicle.¹⁰⁾ One can further conclude that, under conditions of the tests, the solvents are not exerting an influence on the barrier properties of the skin. Among the three vehicles, PG is the only one generally considered to be a skin penetration enhancer. Clearly, no specific enhancement by PG was observed.

Among the three alkyl esters studied, only Ac-BUP showed a significantly higher flux ($p < 0.05$) through the hairless mouse skin than the parent drug, BUP (Fig. 3). Though the difference between Bu-BUP's flux and BUP's flux is not different with a high degree of statistical certainty, the flux of this ester is actually less from the respective saturated solutions, in spite of its one order higher activity. Compared with Bu-BUP, Isb-BUP exhibited a significantly higher skin flux ($p < 0.05$), and it is obvious that it is on a par with BUP's flux. At best, Isb-BUP offers only a slight improvement in total delivery. It is clear from this that solubilities in the skin's conduit phase, presumably intercellular lipids, which cannot be directly measured, are intermediate between what's seen in the polar and nonpolar solvents. This phase must exhibit a polarity closer to that of the polar solvents, for one does not experience even a small fraction of the 66-fold increase in skin flux at Isb-BUP that is suggested by OIL solubilities alone. This observation is just no more small bit of evidence attesting to the fact that the intercellular lipids are substantially polar.

Permeability coefficients of all four drugs were obtained experimentally (Fig. 4) through Eq. 2 using the values of fluxes and solubilities in the respective vehicles. Due to the unique solubility of each drug in each different vehicle, and almost identical skin fluxes, the permeability coefficients mirror solubility relatively accurately (as reciprocals). As a consequence of the solubility trends, the permeabilities from OIL drop systematically as the alkyl chain is extended. From PG and PEG 400, there is an up and down pattern in the permeability coefficients with increased alkyl chain length which also is a reflection of the patterns in solubility. There are two points which should be made about this. First, in structure-activity analysis, permeability coefficients only have meaning if determined from the same event. Second, correlating to this, fluxes from a standardized activity medium only are what are useful in deciding which vehicle offers the best chance of delivering a specified drug in adequate amounts for transdermal or other purposes. While these are not totally new points of view, rarely have they been as well illustrated as in this work.

After each permeation experiment, drug was extracted from the skin to determine the drug accumulation and extent of parent drug regeneration in the skin. As shown in Fig. 5, the amount of ester detected in the skin is roughly proportional to the flux of each drug. It is clear that considerable metabolism of the prodrug occurs within the skin, since both the prodrug and the parent drug were detected in the skin for all three esters. However, all drugs which permeated through the skin appeared as regenerated parent drug in the receptor compartment, except for a small amount of intact ester that was observed for Ac-BUP. These potential prodrugs are highly

Fig. 4. Permeability Coefficients of BUP and Its Alkyl Ester Prodrugs Following Applications of Saturated Slurries in Three Kinds of Vehicles Through Hairless Mouse Skin

PG (closed bar), PEG 400 (open bar), and OIL (hatched bar).

Fig. 5. Drug Disposition in Hairless Mouse Skin after a 48 h Permeation Experiment Following Application of Drugs as a Saturated Slurry in Light Mineral Oil

Each bar represents the concentration of regenerated parent drug or BUP (closed bar) and intact prodrug (open bar) detected in the unit weight of skin tissue. Vertical bars indicate the S.D. of three experiments.

stable in these vehicles and in the aqueous media (unpublished data). Their conversion to the parent compound thus takes place exclusively in the skin during their transit through the tissue. To further investigate this conversion process, skin disposition profiles of the drugs within the skin following the permeation experiment were analyzed. The molar percentages of regenerated parent drug to whole drug collected from the skin following permeation experiments were: Ac-BUP, 9.2%; Bu-BUP, 40.7%; and Isb-BUP, 9.6%, respectively. Comparison of the ratios, $\text{Ac-BUP} \approx \text{Isb-BUP} < \text{Bu-BUP}$, suggests that the regeneration rate of esters to the parent drug is dependent on both the alkyl chain length of the ester (Ac-BUP vs. Bu-BUP) and the structure of the alkyl chain portion of the molecule (Isb-BUP vs. Bu-BUP). In human skin homogenates, Bu-BUP showed a faster regeneration rate (0.017 min^{-1}) than Isb-BUP (0.010 min^{-1}) (unpublished data). This data further supports former contention. There is little doubt that the regeneration process occurs enzymatically in the skin.

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