An *in Vitro* Study of Diamorphine Permeation Through Premature Human Neonatal Skin

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The permeation kinetics of diamorphine through human premature neonatal cadaver skin over a range of gestational ages between 24 and 36 weeks was investigated using small diffusion cells. A strong inverse correlation was noted between the apparent permeability coefficient and the gestational age of the skin ($P < 0.01$; $n = 26$). The calculated apparent permeability coefficients decreased with gestational age from 6.0×10^{-2} cm · hr^{-1} at 24 weeks' gestation to 5.2×10^{-6} cm · hr^{-1} at 36 weeks' gestation. The amount of diamorphine remaining bound within the skin at the end of the *in vitro* experiments did not change significantly with gestational age of the skin. Diamorphine was subject to degradation over the course of the *in vitro* experiments to produce significant amounts of 6-monoacetylmorphine and evidence is presented to suggest that this was due to residual skin esterase activity. It is calculated that the steady-state flux rate of diamorphine through neonatal skin observed in these experiments would be sufficient to obtain a therapeutic plasma concentration of morphine assuming a 2-cm^2 area for application and a delivery rate of 15 μg hr^{-1} kg^{-1}. However, the prolonged half-life of morphine in the premature neonate would result in a delay of some hours before the attainment of this level.

KEY WORDS: diamorphine; percutaneous; transdermal drug delivery; human neonatal skin; premature newborn; skin esterases.

INTRODUCTION

Percutaneous absorption has become accepted in recent years as a potentially important route for the systemic delivery of drugs (1). The major limitations to the permeation of drugs through the skin are the physical barrier of the stratum corneum and the drug metabolizing capacity of the epidermis. In the newborn infant the stratum corneum does not mature until the last few weeks of gestation, and as a result premature babies have an incomplete skin barrier.

The interest in percutaneous penetration through neonatal skin has most often been related to the observed toxic effects of some topically applied compounds used in neonatal intensive care (2). However, it has been suggested that the reduced barrier function of the premature newborn may be turned to therapeutic advantage (3,4). Drugs that would not normally be considered as candidates for transdermal delivery in the adult due to their low rate of penetration may pass through immature neonatal skin at an increased rate, permitting successful drug therapy (5).

Analgesic drugs are increasingly being used on a routine basis for neonatal therapy (6) and form a group of compounds with physicochemical and pharmacokinetic properties which are suitable to permit permeation through skin (7). Indeed it has been shown that certain analgesics (fentanyl, buprenorphine, sufentanil, and alfentanil) have sufficient rate of penetration through skin to be considered as candidates for transdermal delivery to adults (8). A transdermal delivery system has been developed recently for fentanyl pain therapy in man and it has been shown to achieve effective and safe analgesia in clinical trials (9).

In the present study the percutaneous penetration of the opioid analgesic diamorphine through human neonatal cadaver skin has been studied in order to evaluate its potential for transdermal delivery. Because of the known variation in skin barrier function in the premature newborn, the permeation of diamorphine was studied in cadaver skin from newborn infants of gestational ages between 24 and 36 weeks. Diamorphine was the drug of choice because it is used widely in the neonate in the United Kingdom and because data are available on the pharmacokinetics of this drug in the premature newborn (10,11).

EXPERIMENTAL

Materials

Diamorphine hydrochloride and morphine hydrochloride were purchased from the Boots Co. Ltd., Nottingham, UK. 6-Monoacetylmorphine was supplied by Macfarlan-Smith Ltd. (Scotland). All other chemicals were of analytical grade or better.

Skin Preparation

Excised premature neonatal cadaver skin was used in the permeation studies. Samples of whole skin 2–3 cm^2 in area were removed from the abdomen of cadavers within 24 hr postmortem. The skin samples were flattened, wrapped in aluminium foil, sealed in a small plastic envelope, and stored in a freezer at −30°C. The adult human skin samples were abdominal and obtained from cadavers within 24 hr postmortem. The epidermal layer of adult skin was separated from the whole skin by suspension in water (60°C, 2 min), dried at room temperature (30 min), and stored frozen at −30°C prior to use. The frozen skin sections were thawed at room temperature and rehydrated in phosphate-buffered saline (pH 5.6, with 0.01% sodium azide to prevent bacterial growth) at 4°C for 18–24 hr before the experiment. The subcutaneous layer of fat was carefully trimmed from the neonatal skin and the intact skin was mounted into the diffusion cell apparatus for the *in vitro* study.

Skin Permeation Method

Each skin section was mounted carefully between the half-cells of a side-by-side diffusion cell, each of 3.4-mL capacity and with a diffusional area of 0.64 cm^2 (Crown Glass Company, USA). The two half-cells were firmly clamped

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together and the receptor half-cell (epidermal side) was filled with buffer (PBS, pH 5.6) and the donor half-cell (stratum corneum side) was filled with diamorphine solution (2.5 or 10 mg · mL^{-1} in 0.1 *M* acetate buffer, pH 4.0). The acid pH was used to maintain the stability of diamorphine, which is rapidly degraded at neutral pH, and also to assist with the solubility of the drug in water. The jacketed diffusion cells were maintained at 32°C using a flow heater (Grant Instruments, Cambridge, UK) and each half-cell was stirred throughout the experiment at approximately 100 rpm. At predetermined times 0.2-mL aliquots of the receptor content were withdrawn and replaced with equal volumes of fresh buffer. Samples (0.2 mL) were taken from the donor compartment at the beginning of each experiment and at the end of each day of the study to ensure that the depletion of the drug did not exceed 10% during the course of the study. At the end of the experiment the skin was removed from the apparatus and rinsed twice in pH 5.6 buffer, blotted dry, and then extracted in methanol for 24 hr to remove any diamorphine remaining bound to the skin. The permeation samples and the skin extracts were analyzed by high-performance liquid chromatography (HPLC) for the concentrations of diamorphine, 6-monoacetylmorphine, and morphine.

Analytical Methods

Diamorphine, 6-monoacetylmorphine, and morphine were analyzed by HPLC under the following conditions: The column was a Spherisorb (Phase Separations, Queensferry, UK) ODS 1, 15 cm long, 4.6 mm in internal diameter, with a 3-μm particle size; the mobile phase comprised 65% potassium phosphate buffer (0.05 *M*), 35% acetonitrile, and 0.1% triethylamine, adjusted to pH 3.0 with orthophosphoric acid. The flow rate was 1.0 mL min^{-1} and the injection volume was 50 μL. The limit of detection of each compound was 0.05 μg · mL^{-1} and the assay was linear over the range 0.05 to 100 μg · mL^{-1}.

Assessment of Stability of Diamorphine

Diamorphine stability was assessed at concentrations of 10 mg · mL^{-1}. A freshly made-up solution of the compound, in 0.1 *M* acetate buffer (pH 4.0) and 0.1 *M* phosphate buffer (pH 5.6) as appropriate, was transferred to a jacketed glass vessel or test tube maintained at 32°C. Samples (0.2 mL) were removed from the vessel at appropriate time intervals and analyzed by HPLC for the concentration of diamorphine, 6-monoacetylmorphine, and morphine.

Analysis of Data

The data were plotted as the cumulative amount of drug collected in the receptor cell versus time. The apparent permeability coefficient for a given run was calculated from Fick's first law:

$$J_T = A \cdot P \cdot \Delta C$$

In this equation J_T is the total steady-state flux of drug in μg · hr^{-1}; *A* is the area of the membrane (0.64 cm^2); *P* is the apparent permeability coefficient in cm · hr^{-1}; and ΔC is the concentration difference between the two chambers of the diffusion cell, which was taken as the initial donor phase concentration in μg · mL^{-1}. The steady-state flux was calculated from the slope of the terminal, linear portions of the plot of cumulative diamorphine permeation versus time using linear regression analysis. The lag time was calculated as the intercept of the steady-state portion of the curve on the time axis. Diamorphine is present in the donor solution predominantly in its ionised form (pK_a = 7.83) and therefore the calculated permeability coefficients relate to the diffusion of the ionized species.

Pharmacokinetic simulations were performed with the PCNONLIN software package (Statistical Consultants Inc., Lexington, USA) based on a 1.0-kg-birth weight infant, a half-life of 8.9 hr, and a volume of distribution of 2.7 L for morphine (10).

RESULTS AND DISCUSSION

Kinetics of Diamorphine Permeation

The details of the skin samples, the apparent permeability coefficients, steady-state fluxes, and lag times of diamorphine penetration through neonatal cadaver skin and adult skin are summarized in Table I. Representative plots of the cumulative amounts of diamorphine permeated through human premature neonatal cadaver skin as a function of time for different gestational ages are shown in Fig. 1. A pseudo steady-state flux of diamorphine was achieved with all the skin samples, although the restricted axes in Fig. 1 do not necessarily show this stage for all the skin samples. The relationship between gestational age of the premature neonatal skin and apparent permeability coefficient is shown in Fig. 2, which demonstrates a strong inverse correlation between these two variables ($P < 0.01$, $n = 26$). There is a factor of 10^4 difference between the apparent permeability coefficient of 6.0×10^{-2} cm · hr^{-1} through extremely premature skin (24 weeks) compared with that of 5.2×10^{-6} cm · hr^{-1} in a nearly full-term skin. Diamorphine penetration through adult epidermis was found to be similar to that in the full term neonate. This observation is in agreement with previous data showing a significant increase in permeation rate for premature skin compared with full-term infant skin or adult skin. McCormack *et al.* (12) found that alcohol penetration was 50 times greater through preterm skin compared with mature skin and Barker *et al.* (13) noted that salicylate absorption was up to 1000 times greater at 26 weeks' gestation than at full term. The present study provides further and more comprehensive evidence of the considerably reduced barrier to drug penetration in neonatal skin. The changes in permeability of the skin appear to correlate well with histological studies of the development of the epidermis and stratum corneum in the newborn (14,15). In these studies gestation was found to have a marked influence on epidermal development. Prior to 30 weeks' gestation the epidermis is thin and poorly formed, and little or no stratum corneum is present, but by 34 weeks it has largely matured and is histologically indistinguishable from adult skin. The permeation data in our study reflect these observed changes in the histology of premature neonatal skin and suggest a slow development of the skin barrier to reach full maturity between 32 and 34 weeks' gestation.

Table I. Data on the *in Vitro* Permeation of Diamorphine Through Premature Human Neonatal Skin

Gestational age (weeks)	Postnatal age (days)	Apparent permeability coefficient (cm/hr × 10^{-4})[a]	Flux rate (μg/cm²/hr)[a,b]	Lage time (hr)[a]	Diamorphine bound to skin (μg/cm²)[a]
24	1	540	140	<0.50	
24	2	300	300	0.93	
25	3	270	270	<0.50	15.3
26	s/b[c]	52	19.5	0.51	
27	3	9.8	9.8	0.68	
28	2	35.5	7.8	13.30	
28	2	2.83	2.8	<0.50	12.1
29	12	2.20	2.2	0.97	
31	3	1.48	1.5	<0.50	
34	3	2.48	2.5	<0.50	
36	3	0.071	0.18	<0.50	
38	26	0.23	0.23	8.00	19.3
40	7	0.077	0.08	14.00	14.1
Adult cadaver skin epidermis	68 years	6.7	0.07	35.00	9.8

[a] Mean of two determinations.
[b] Flux rates determined as diamorphine equivalents.
[c] Stillborn.

Diamorphine Stability Under Experimental Conditions

Diamorphine was quantitatively deacetylated in aqueous acetate or phosphate buffer saline at 32°C to give 6-monoacetylmorphine as the sole degradative product. The half-life of diamorphine under these conditions was calculated to be 13 days in the receptor phase (phosphate buffer, pH 5.6) and 42 days in the donor phase (acetate buffer, pH 4.0). The degradation was found to obey first-order kinetics. Most of the experiments on the *in vitro* permeability of diamorphine were complete within 72 hr and the degree of breakdown of the drug, based on the measured rate constants, would therefore be expected to be minimal. However, under the conditions of the *in vitro* permeation experiments, it was found that 68.6 ± 12.7% (mean ± SD; $n = 10$) of the diamorphine in the receptor phase had been degraded to 6-monoacetylmorphine after 72 hr. From these results it was reasoned that residual esterase activity on the epidermal side of the neonatal skin was responsible for the increased rate of degradation of diamorphine compared with that in aqueous solution. It should be noted that this epidermal esterase activity may be due to the leakage of enzymes from the skin caused by cell damage during the skin preparation and storage. However, a similar enzymic degradation of morphine esters during *in vitro* experiments with adult human skin has been reported by Fullerton *et al.* (16). This enzymic hydrolysis of diamorphine by skin is not necessarily a disadvantage since diamorphine itself is pharmacologically

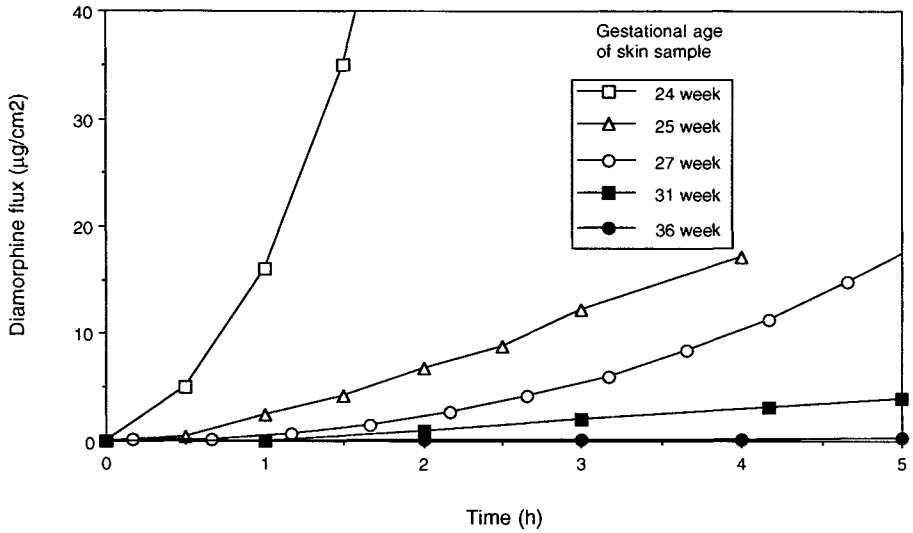

Fig. 1. The effect of gestational age on the cumulative flux of diamorphine through premature neonatal skin.

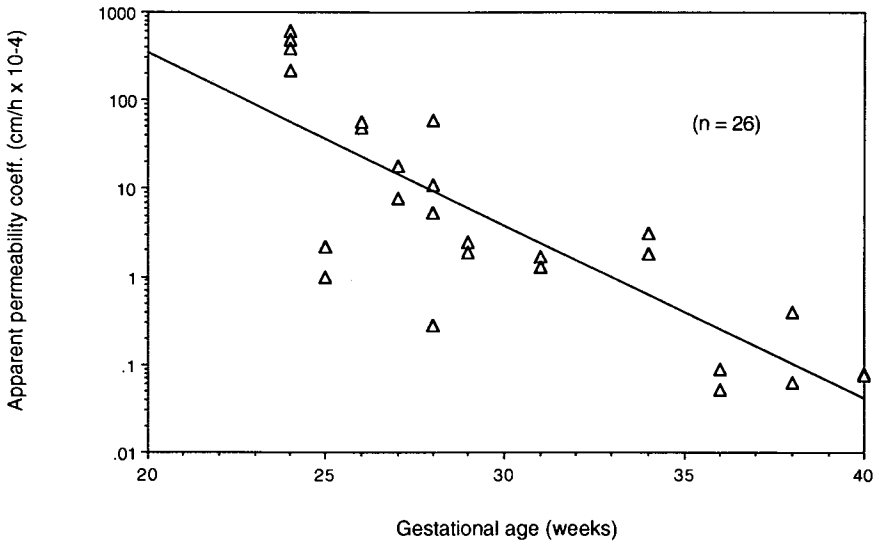

Fig. 2. The relationship between gestational age and diamorphine permeability through premature neonatal skin.

inactive and it is the metabolites of diamorphine, 6-monoacetylmorphine, morphine, and morphine-6-glucuronide which mediate the analgesic action of the drug. Thus diamorphine may be considered a prodrug with physicochemical properties suitable for transdermal delivery of its active metabolites.

Potential Transdermal Delivery of Diamorphine to the Neonate

Based on a mean plasma clearance value of 3.6 mL min^{-1} kg^{-1} for morphine (the main active metabolite of diamorphine) in the premature neonate and a known therapeutic steady-state morphine plasma concentration of 62.5 ng mL^{-1} (10), it is possible to calculate the desired transdermal delivery rate of morphine to be 13.5 μg hr^{-1} kg^{-1} using a standard pharmacokinetic formula (17). Assuming complete conversion of diamorphine to morphine, then this is equivalent to a diamorphine infusion rate of 15 μg hr^{-1} kg^{-1}. A practical skin area of 2 cm^2 for the clinical application of a transdermal patch would result in a desired flux rate of 7.5 μg hr^{-1} kg^{-1} across the skin. This value is well within the reported flux rate of diamorphine through neonatal skin of gestational age less than 29 weeks. However, since this flux rate was obtained with an unsaturated solution of diamorphine at a pH unlikely to give optimum penetration of diamorphine, it is probable that the desired flux rate could readily be obtained in neonates of gestational age up to 34 weeks. It should be noted that over 85% of babies requiring pain relief in the Nottingham neonatal intensive care units have gestational ages of between 24 and 32 weeks (unpublished data, N. Rutter, 1990). These calculations do not take into account recent evidence suggesting that morphine-6-glucuronide, a metabolite of morphine, also has powerful analgesic activity (18).

A further consideration relating to the transdermal delivery of an analgesic such as diamorphine is the time to reach an effective plasma concentration after administration of the drug. This may be readily calculated from the known mean half-life of morphine in the premature newborn of 8.9 hr (10) and indicates that a delay of greater than 24 hr would be anticipated with constant-rate transdermal drug delivery. A slow attainment of steady-state blood concentration due to a prolonged drug half-life was also found *in vivo* by Evans *et al.* (19) for the transdermal administration of theophylline sodium glycinate to the neonate. A delay in reaching steady-state blood concentration following transdermal delivery is likely to apply generally to premature infants since many drugs have a considerably prolonged half-life in this age group.

The resulting delay in onset of action of analgesia with transdermal diamorphine therapy can be easily overcome by the administration of a intravenous loading dose of diamorphine to achieve the rapid attainment of a therapeutic level

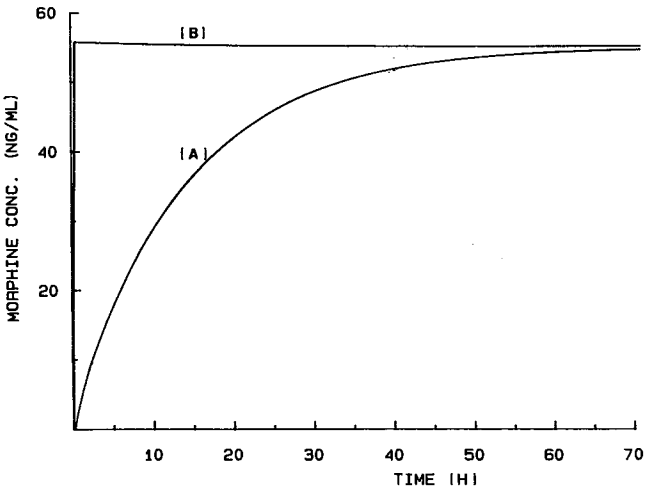

Fig. 3. Pharmacokinetic simulation of morphine plasma concentration–time profile resulting from (A) a constant-rate transdermal delivery of diamorphine at 15.0 μg/kg/hr and (B) a 200-μg/kg intravenous loading dose followed by a constant-rate transdermal delivery of diamorphine of 15.0 μg/kg/hr.

of morphine which can be subsequently maintained by transdermal delivery (Fig. 3). While detracting somewhat from the noninvasive ideal of transdermal delivery to the neonate, this would be a practical procedure, since normally all premature babies in neonatal intensive care are fitted with arterial or venous indwelling cannulae which readily permit the administration of an intravenous bolus of a drug. The potential advantage of transdermal delivery in this case would be the elimination of the need for the insertion of an intravenous cannula, which is often a painful and disruptive procedure in the premature newborn.

In conclusion, the data on the *in vitro* permeation of diamorphine through premature neonatal skin indicate that this drug may be suitable for transdermal delivery to this subject group in intensive care.

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