Porcine Ear Skin as a Model for the Assessment of Transdermal Drug Delivery to Premature Neonates

Nabila Sekkat,[1,2,3] Yogeshvar N. Kalia,[1,2] and Richard H. Guy[1,2,4]

Received March 16, 2004; accepted April 9, 2004

Purpose. The purpose of this study was (i) to validate differentially tape-stripped, porcine skin as an *in vitro* model for the evaluation of transdermal drug delivery (TDD) to premature neonates, (ii) to determine whether the model could estimate neonatal skin permeability as a function of postconceptional age (PCA), and (iii) to demonstrate that iontophoretic delivery permits precise control of drug input independent of skin barrier function.

Methods. Passive permeation of caffeine, phenobarbital, and lidocaine across tape-stripped porcine skin barriers was measured. Iontophoretic delivery of lidocaine across skins with different barrier competencies was also evaluated.

Results. For all drugs, passive permeation correlated with skin barrier function; that is, with transepidermal water loss (TEWL): $J_{ss} = A \cdot \exp[B \cdot TEWL]$. Combining this result with a previously derived dependence of TEWL upon the PCA of premature neonates *in vivo* allowed a relative value of J_{ss} to be predicted for a given PCA. Comparison of these predictions showed excellent agreement with experimental data reported for diamorphine. Iontophoretic lidocaine delivery was precisely controllable independent of barrier competency.

Conclusions. Porcine skin, *in vitro*, differentially tape-stripped to specific barrier competencies, is a useful model to explore TDD in premature neonates. The potential for iontophoresis to provide improved dose control and adjustment, irrespective of skin barrier maturity, is established.

KEY WORDS: iontophoresis; premature neonatal skin; porcine skin; skin barrier function; tape stripping; transdermal drug delivery; transepidermal water loss.

INTRODUCTION

Premature neonates represent a vulnerable patient population for whom it is necessary to develop noninvasive routes of drug administration. In effect, premature neonates endure several sequelae (1–3) issuing from their immaturity (e.g., apnea, seizures, cardiovascular problems), and hence are subject to multiple-drug therapies. Indeed, approximately 99% of neonates weighing less than 1.5 kg routinely receive a multiple-drug administration consisting of antibiotics, analgesics, cardiovascular, respiratory, and nutritional agents (4,5). These treatments are predominantly administered parenterally, a procedure that is very painful and stressful for the preterm neonates and that often leads to inadequate healing at the injection sites. In addition, because of the very low drug doses usually administered, small variations in infusion rate may lead either to a lack of therapeutic efficacy or to toxic effects (4). Oral drug administration as an alternative can be problematic, as absorption from the immature gastrointestinal tract of the premature neonates is quite unpredictable. Moreover, as the neonate matures, gastrointestinal motility and gastric emptying time change dramatically leading to significant variability in drug absorption (6).

In contrast, transdermal drug delivery represents a potentially noninvasive, continuous, and controllable alternative for the premature neonate. Premature neonatal skin constitutes a unique opportunity for this route of administration, because the cutaneous barrier function is underdeveloped, and therefore more permeable than the fully mature organ (1,4,7). Several studies have already investigated the transdermal application of drugs, such as theophylline (8,9) and caffeine (10), to the underdeveloped premature neonatal skin. In these studies, therapeutic concentrations were achieved in premature neonates *in vivo*. However, drug absorption significantly decreased as the neonatal age increased, and skin maturation occurred. Therefore, because the skin of the neonate is a "moving target" that changes and develops after birth (11), a fundamental understanding of the evolution of skin permeability at any given gestational and postnatal age is essential for the development of transdermal delivery applications. A method to predict changes in the permeability of premature skin would permit an appropriate delivery rate of drug to be matched to the gestational and postnatal age of the neonate.

Previously, X-ray diffraction, infrared spectroscopy, light and electron microscopy, and high-performance thin layer chromatography have been used to characterize structurally and compositionally the intercellular lipids of neonatal stratum corneum (i.e., the outermost skin layer) as a function of gestational and postnatal age (12). More recently (2), the functional development of a complete barrier has been evaluated by measurements of transepidermal water loss (TEWL), control of which represents skin's principal function. A body of information exists, therefore, with which to "calibrate" the permeability of infant skin. Thus, it is hypothesized that TEWL, a simple biophysical and functional measurement of neonatal stratum corneum, is predictive of the permeability barrier, and, as such, can be used to develop safe and effective transdermal drug delivery systems. This is supported by the fact that, for instance, Cartwright *et al.* have demonstrated a strong correlation between maximum plasma theophylline concentration and TEWL in premature neonates (9).

Clearly, ethical and safety concerns do not allow the permeability of neonatal skin to different drugs to be directly tested *in vivo*. Furthermore, the most relevant *in vitro* model, excised skin from neonates of different gestational and postnatal ages, is simply not available in any significant quantity. What is possible, however, is that one can "create" [using excised porcine skin, which is generally recognized as an excellent approximation of the human equivalent (13)] barriers of varying competency, from 0 to 100% function, quantify precisely this functionality by TEWL, and then, in turn, quantify the permeability of different drugs across the tissue. In

[1] School of Pharmacy, University of Geneva, CH-1211 Geneva 4, Switzerland.

[2] Centre Interuniversitaire de Recherche et d'Enseignement, Universités de Genève et Lyon, "Pharmapeptides," Parc d'Affaires International, F-74166 Archamps, France.

[3] Novartis Pharma AG, WSJ-340.701, Postfach 4002 Basel, Switzerland.

[4] To whom correspondence should be addressed. (e-mail: Richard.Guy@pharm.unige.ch)

0724-8741/04/0800-1390/0 © 2004 Plenum Publishing Corporation

previous work (14), measurements of TEWL across a porcine ear skin model *in vitro* (which was physically impaired by progressive tape-stripping) were compared with TEWL data of premature neonatal skin *in vivo* (2). Remarkable similarities were found between the *in vitro* model and premature neonatal skin *in vivo*. Thus, a demonstration that TEWL across the porcine ear skin model correlates directly with drug permeability would then provide a tool with which transdermal dosing to neonates at different stages of development could be tailored and optimized. In this study, three clinically relevant drugs for premature neonates (4), caffeine (10) (i.e., against apnea), phenobarbital (15) (i.e., against epileptic seizures), and lidocaine, a local anesthetic, have been used to validate this hypothesis.

In practical terms, though, it is apparent that the use of passive transdermal drug delivery will not be entirely straightforward. First, there is the question of inter-and intrasubject variability in skin permeation, at no matter what the level of stratum corneum (SC) development (and this seems to be greater as the barrier evolves), an issue of considerable importance for drugs having a narrow therapeutic window. Second, as the SC develops, and its permeability decreases, it will be necessary to find a way to maintain the dose delivered per application (or even perhaps to increase it given that the neonate will be gaining weight). The simplest approach, of course, is to increase the area of application progressively, but this then raises questions of how the delivery system should be formulated and applied (i.e., use of patches of different size vs. more conventional semisolid vehicles, the administration of which is not easy to standardize).

Ideally, then, one requires the transdermal (i.e., noninvasive) equivalent of a simply adjustable intravenous pump and, of the technologies presently available, iontophoresis offers a viable option. In iontophoresis, the application of a small current between two electrodes in contact with the skin can be used to deliver ionic drugs (and even neutral compounds) at much greater levels than passive diffusion (16). However, the elegance of the method resides in the fact that the charge (in Coulombs) delivered by the power supply of the iontophoretic device exactly equals the charge that flows across the skin and that is carried by endogenous ions moving out of the body and by ions (including the drug) from the electrode formulations moving into and through the skin (17,18). Hence, whereas drug delivery by iontophoresis cannot be 100% efficient, as the drug must compete with other ions in the system to carry charge across the skin, it is very reproducible and very controllable: if the current delivered by the power supply is doubled, then (as long as the fraction of charge carried by the drug, i.e., its transport number, remains constant) the amount of drug transported will likewise increase by 2-fold; if the current is reduced by one-third, a similar reduction in drug delivery will result; if the current is stopped, drug movement is limited to its passive transport rate—in other words, at least for ionized species, it also ceases. Because of this sensitivity, it follows furthermore that iontophoretic delivery is much less sensitive to the nature of the skin barrier (again, with the caveat that the transport number of the ion of interest remains relatively constant). Indeed, the literature shows clearly that interspecies differences in passive permeability to drug diffusion are remarkably normalized when iontophoresis is applied (19). This is because, of course, as we have described above, it is the quantity of charge provided by the power supply that determines the quantity of ions that move across the skin.

We postulate, therefore, that drug delivered by iontophoresis across skin barriers of different functionalities should be controllable provided (a) that passive diffusion constitutes a relatively unimportant component of the total flux and (b) that the drug's transport number across the differentially efficient barriers remains reasonably constant. If this hypothesis is shown to be correct, it would follow that iontophoresis may offer a unique and useful drug delivery alternative to the premature neonate. Therefore, in the second part of this investigation, we test this idea using lidocaine as a model drug, and one whose passive permeation across premature neonatal skin has already been reported (20).

MATERIALS AND METHODS

Skin Preparation

Porcine ears were obtained immediately post-sacrifice from a local abattoir (Annecy, France). The skin was gently cleaned and then excised. Fat was removed from the dermal surface and the tissue from each ear was divided into four pieces of approximately 4 cm^2. The first piece (fully intact skin) was not subjected to further treatment; a second piece was tape-stripped repeatedly, as previously described (14), to provide a completely deranged (i.e., no stratum corneum) barrier. Transepidermal water loss (TEWL) measurements, performed with a commercial apparatus (Servomed EP1, Servomed AB, Varberg, Sweden), were recorded throughout the stripping procedure and clearly showed when no further SC remained to be removed. The third and fourth pieces of skin were tape-stripped to two intermediate levels of barrier function (nominally representing of the order 60–80% and 20–40% of the intact SC). Again, TEWL measurements were used to characterize the precise levels of barrier functionality achieved. The skin samples prepared in this way provided membranes with a range of barrier function from 0 to 100% for a series of passive diffusion experiments and a series of iontophoresis studies.

Passive Permeation

The permeation of caffeine (Aldrich, St-Quentin, France), lidocaine hydrochloride (ICN Biomedicals, Aurora, OH, USA) and phenobarbital (Sigma, St Quentin, France) across the four barriers of different efficiencies was measured in vertical, flow-through diffusional cells (area of skin = 3.14 cm^2) thermostatted at 35°C. The donor formulations (3 ml) were aqueous solutions of caffeine and phenobarbital, at concentrations close to saturation (20 g/L for caffeine, 1 g/L for phenobarbital), for which >99% of the permeant was unionized. The magnetically stirred receptor phase (6 ml) was phosphate-buffered saline at pH 7.4 and was continuously perfused, such that samples could be collected hourly on a fraction collector for up to 24 h. In the case of lidocaine, the experimental conditions selected were based on those used previously in studies with premature neonatal skin. The donor was an aqueous solution (3 ml) of the drug at a concentration of 10 mg/ml; the solution was not buffered and had a pH (4.8) reasonably close to that (pH 4) used in the earlier neonatal skin study (20). The magnetically stirred receptor

phase (6 ml) contained phosphate-buffered saline at pH 5.6 and was perfused continuously, allowing fractions to be collected hourly for up to 15 h.

Iontophoresis

Electrotransport of lidocaine was similarly studied across the different skin barriers produced by the tape-stripping protocol. Vertical cells, specifically designed for iontophoresis (21), were used. The anode compartment contained the same drug solution as used in the passive experiments; the cathode and receptor solutions were phosphate-buffered saline at pH 5.6. The electrodes were Ag/AgCl and were connected to a computer-controlled power supply that provided a current of 0.3 mA/cm^2. The experimental duration was 6 h, during which time samples of the receptor phase were collected every 30 min.

Assays

The samples were filtered through nylon membranes (0.45 μm, Nalgene syringe filters) and were then assayed for permeated drug by high-performance liquid chromatography (Waters 600 Controller pump, Autosampler injector 717-plus, and In Line Degasser, Waters, St-Quentin Yvelines, France), with UV detection (Waters 486 Tunable Detector) (22,23).

Caffeine was detected at 273 nm using a Novapak C18 column (30 cm × 3.9 mm, Waters); the mobile phase was 20% acetonitrile, 0.1% diethylamine in water, and 79.9% deionized water, and the pH was adjusted to 2.5. The flow rate was fixed at 1.0 ml/min. Phenobarbital was analyzed at 215 nm, using a Supelcosil C18 column (25 cm × 4.6 mm, Supelco, Saint Quentin Fallovier, France). The mobile phase contained 30% acetonitrile and 70% phosphate buffer (0.067 M KH_2PO_4), and the pH was adjusted with NaOH to 6.0. The flow rate was 2.0 ml/min. All chemicals used in the assay procedures were acquired commercially (Sigma-Aldrich, St-Quentin, France) and were of at least analytical grade. Lidocaine was detected at 220 nm using a Supelcosil LC-18 column (25 cm × 4.6 mm, Supelco). The mobile phase was 25% acetonitrile, 74.9% phosphate buffer (0.067 M KH_2PO_4), and 0.1% triethylamine. The pH was adjusted with HCl to 3, and the flow rate was 1.0 ml/min. Lidocaine had a retention time of 5.6 min.

Data Analysis

The passive results were expressed as the cumulative amount of drug transported across the skin barrier per unit area (Q) as a function of time (t). Each permeation curve was fitted to the appropriate solution (Eq. 1) of the nonsteady-state diffusion equation [Fick's second law (24)], which assumes the boundary conditions (a) that there is no depletion of the drug in the donor compartment over the course of the experiment, (b) that the receptor phase provides "sink conditions" (25), and (c) that at t = 0, there is no drug in the skin. That is,

$$Q = \{K\ H\}\ C_{veh}\left[\frac{D}{H^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{H^2}\right)\right] \quad (1)$$

Where C_{veh} is the drug's concentration in the donor solution and K is its SC-solution partition coefficient; D is the diffusivity of the drug in the SC of thickness H.

The fitting procedure used a commercial software package (Kaleidagraph, Version 3.0.5, Synergy Software, Reading, Pennsylvania, USA), running on a personal computer, and enabled the drug's characteristic partitioning (KH) and diffusivity (D/H^2) parameters to be deduced. In turn, the classic permeability coefficient (K_p) of the drug across the skin could be deduced:

$$K_p = (K\ H) \times \left(\frac{D}{H^2}\right) = \frac{K.D}{H} \quad (2)$$

together with the estimated steady-state drug flux J_{ss}

$$J_{ss} = K_p \times C_{veh} \quad (3)$$

RESULTS AND DISCUSSION

As expected, the differentially tape-stripped pieces of porcine ear skin demonstrated markedly different permeabilities. Figure 1 illustrates the results for lidocaine. Though this ionized drug penetrates a complete barrier very poorly, it is absorbed over 100 times more easily when the SC has been removed. As expected, barriers of intermediate functionality have permeabilities that fall between the two extremes. Complete derangement of the barrier, of course, permits facile and rapid diffusion of the permeant. The point is reinforced in Fig. 2, which shows, respectively, the permeation profiles of the caffeine and phenobarbital across fully intact and completely deranged barriers. Removal of the SC enabled caffeine penetration to increase on average by 33-fold relative to its transport across intact skin; the corresponding increase (19 times) was of similar order for phenobarbital. Less variability in permeability between skin samples without SC was apparent, compared to those of full functionality, a finding again not surprising in that the deranged skin lacks the inhomogeneity associated with different SC thickness, different intercellular lipid composition, and so on.

The partitioning and diffusivity parameters, together

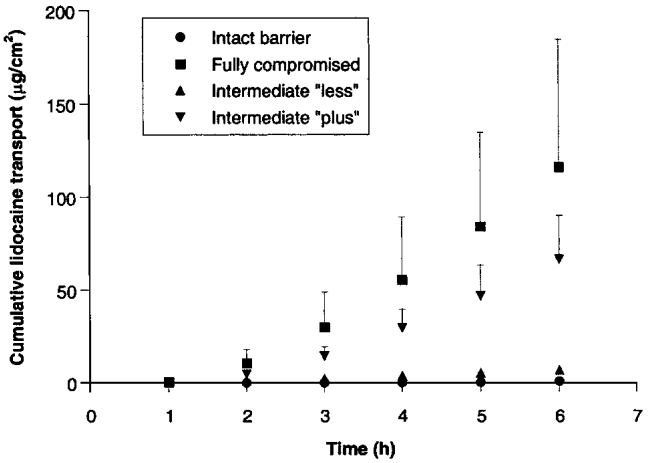

Fig. 1. Representative lidocaine permeation profiles across differentially impaired porcine skin barriers as a function of time. Curve B_{100} corresponds to a completely intact barrier; B_0 to skin that has been completely stripped of its stratum corneum. $B_{60/80}$ and $B_{20/40}$ are barriers of intermediate functionality (with, nominally, 60–80% and 20–40%, respectively, of an intact SC).

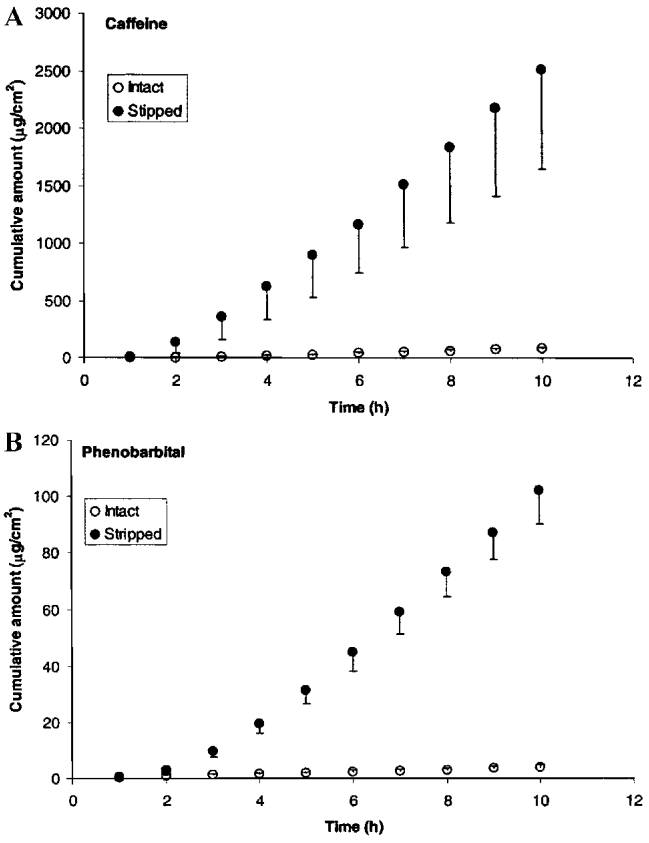

Fig. 2. Cumulative permeation profiles of (a) caffeine (n = 6) and (b) phenobarbital (n = 3), across intact and completey deranged (i.e., no SC) porcine skin as a function of time. Each data point represents the mean ± SD.

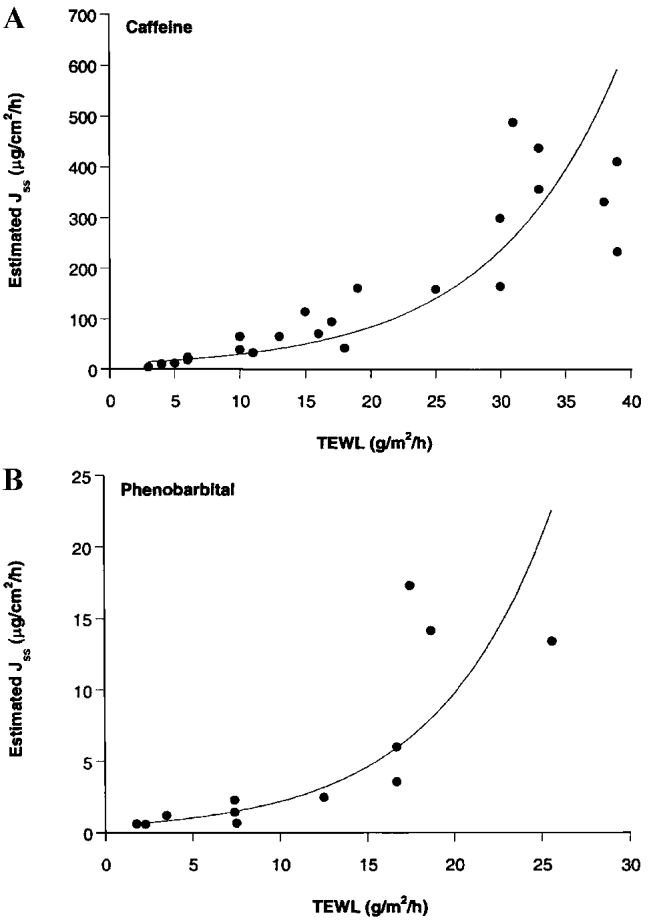

Fig. 3. Estimated steady-state drug fluxes J_{ss} across differentially impaired skin barriers as characterized by the corresponding values of TEWL measured prior to the permeation experiments. (a) Caffeine (n = 24): The empirical fit to the data shown is $J_{ss} = 10.8\ e^{(0.10 \cdot TEWL)}$, with $r^2 = 0.84$. (b) Phenobarbital (n = 12): The empirical fit to the data shown is $J_{ss} = 0.50\ e^{(0.15 \cdot TEWL)}$, with $r^2 = 0.83$.

with the deduced permeability coefficients and steady-state fluxes, of caffeine and phenobarbital across the fully intact and completely deranged barriers are in Table I. It can be seen that the order of magnitude increase in drug flux observed on removing the SC is principally associated with a similar change (i.e., an increase) in the characteristic partitioning and diffusion parameters.

For the skin barriers of intermediate efficiencies, drug transport fell between the extremes of those across fully functional SC and of the SC-free membrane. In parallel, the derived partitioning, diffusivity, and permeability coefficient parameters had intermediate values to those shown in Table I. A summary of these findings is attempted in Fig. 3, which presents, for caffeine and phenobarbital, the dependence of their estimated steady-state fluxes (J_{ss} as determined from Eq. 3) on the functionality of the skin barrier, expressed in terms of the TEWL rate measured subsequent to the preparatory tape-stripping procedure. Drug flux is observed to change slowly at first with the rate of water loss across the skin, but then to increase more rapidly. Interestingly, in the same model, TEWL mirrors a very similar pattern of behavior as a function of SC thickness (14) as does the TEWL rate measured in premature neonates *in vivo* as a function of their postconceptional age (PCA) (see below); that is, when the barrier is absent or nearly so, molecular transport is facile but, as functionality appears, permeability diminishes rapidly.

Empirically, the results in Fig. 3 have been fitted to a simple exponential equation of the form:

Table I. Drug Partitioning (KH) and Diffusivity (D/H^2) Parameters, Together with Estimated Permeability Coefficients (K_p) and Steady-State Fluxes (J_{ss}), of Caffeine and Phenobarbital Across Fully Intact (% Barrier Function = 100) and Completely Deranged (% Barrier Function = 0) Porcine Skin *in Vitro*

Drug	Caffeine (n = 6)		Phenobarbital (n = 3)		Lidocaine HCl (n = 3)	
% Barrier function	100	0	100	0	100	0
KH (cm)	0.01 ± 0.01	0.21 ± 0.07	0.04 ± 0.03	0.28 ± 0.05	0.01 ± 0.004	0.06 ± 0.03
D/H^2 (h^{-1})	0.05 ± 0.03	0.11 ± 0.07	0.03 ± 0.01	0.05 ± 0.003	0.02 ± 0.004	0.06 ± 0.01
$10^4 \cdot K_p$ (cm/h)	5.8 ± 3.5	187.9 ± 44.8	8.1 ± 3.6	149.5 ± 20.7	1.6 ± 0.3	36.0 ± 19.8
J_{ss} ($\mu g \cdot cm^{-2} \cdot h^{-1}$	11.5 ± 7.0	375.7 ± 89.7	0.8 ± 0.4	14.9 ± 2.1	1.6 ± 0.3	36.0 ± 19.8

$$J_{ss} = A \cdot \exp [B \cdot TEWL] \quad (4)$$

Though the pre-exponential factors (A) are, as expected, different for caffeine and phenobarbital, given their different physicochemical properties and the 20-fold difference in C_{veh}, the exponents (B) are quite similar, suggesting perhaps that a particular level of barrier function compromization or normalization will provoke the same *relative* effect on the transport of different substances.

The question posed in this work, then, is whether the relative changes in J_{ss} predicted by Eq. 4, as a function of barrier function (as measured by TEWL), are indicative of what may be observed across the developing skin of premature neonates. Although caffeine and phenobarbital have been administered topically to premature neonatal skin (10,15), it is not possible to re-express the data in the literature in terms of a flux or permeability coefficient, so to make a direct comparison with our findings here. However, perhaps as a better test of the hypothesis, there are published results for the permeation of another drug, diamorphine, across premature neonatal skin (26), which do lend themselves to comparison. From a graphical representation of some of the data on this drug (26), it is possible to estimate the relative flux of the drug across three barriers of different maturity, specifically those from infants of estimated PCA of 178, 192, 220 days. The estimated diamorphine flux ratios J_{220}/J_{178} and J_{220}/J_{192}, are shown in Fig. 4 together with the *predicted* values from the caffeine and phenobarbital experiments reported here. How were the latter determined? In a previous report, we re-analyzed a large body of TEWL measurements performed *in vivo* in premature neonates over a wide range of PCA and generated the following relationship:

$$TEWL = 3.3 + 41 \times \exp [-0.026 \{PCA - 160\}] \quad (5)$$

It follows that, for any given PCA, a value of TEWL can be predicted and then used in Eq. 4 to estimate the corresponding J_{ss}. As we do not have specific data in the porcine ear model for diamorphine, we cannot determine J_{ss} absolutely, but relative values can be found (as this allows the unknown pre-exponent to be cancelled out), and these appear in Fig. 4a.

Given the differences in experimental design, data analysis, and interpretation, the agreement between the observed diamorphine results and the predictions from the *in vitro* porcine model described here is remarkably good, with the phenobarbital estimate doing exceptionally well (perhaps because it is a relatively lipophilic drug like diamorphine). It follows that relationships like those expressed in Eqs. 4 and 5 may be useful for estimating drug delivery across a premature neonate's skin as a function of its PCA. That is, by combining the two functions, one may derive a visual representation of the approach to barrier normalization (see Fig. 4b).

The lidocaine data were analyzed in a similar manner. Figure 5 displays the K_p values deduced as a function of skin barrier efficiency, which is expressed in terms of the TEWL value measured across each membrane after it had been differentially tape-stripped. Consistent with what had been seen for caffeine and phenobarbital, K_p increased exponentially with increasing TEWL. The empirical exponential fit of the data in Fig. 5 ($r^2 = 0.89$) reveals an exponent of 0.13, a value remarkably similar to those obtained for caffeine and phenobarbital.

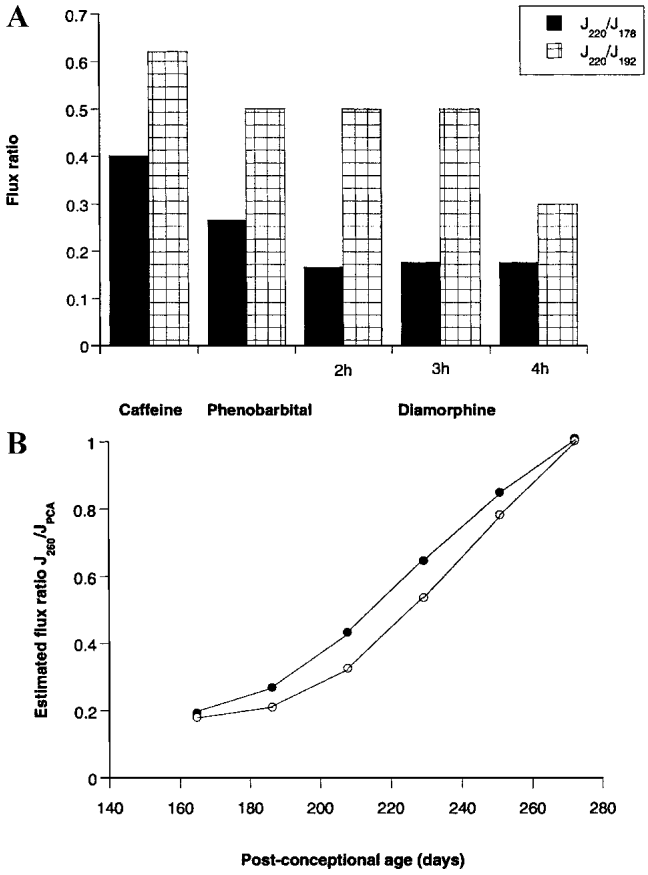

Fig. 4. (a) Predicted flux ratios across premature neonatal skin (a) at 220 days postconceptional age (PCA) compared to 178 days PCA (filled bars), and (b) at 220 days PCA compared to 192 days PCA (hatched bars), based on the caffeine and phenobarbital data reported in this paper (see text for details of these calculations). These predictions are contrasted at right with experimental data for diamorphine, obtained at different times postapplication, as reported in the literature (see Fig. 1 of Ref. 26). (b) Predicted ratios of drug flux across the skin of a full-term infant (J_{260}) to that across the incompletely developed barrier of a premature neonate of postconceptional age less than 260 days (PCA). The upper limit of the estimated ratio is based on the caffeine data reported in this article; the lower limit is based on the phenobarbital results.

Equation 5 was used to re-express the extensive published K_p data for the passive transport of lidocaine across neonatal skin samples of varying PCA (27) in terms of TEWL and then compared with the model *in vitro* results already presented (see Fig. 5). The comparison is shown in Fig. 6 and indicates an impressive overlap between the two sets of data. Because of the wide range of K_p values, the results are displayed semi-logarithmically; simple linear regressions through the points are also shown in Fig. 6 and are remarkably similar.

It follows, therefore, that drug transport across the *in vitro* porcine skin model can be used to both qualitatively and quantitatively predict transdermal transport across the skin of a premature neonate. Knowing the PCA of the infant enables the TEWL rate across its skin (i.e., its barrier functionality) to be deduced from Eq. 5. This value of TEWL can then be applied to the relationship derived from Fig. 5 ($10^4 \cdot K_p = 0.97 \cdot \exp [0.13 \cdot TEWL]$) to estimate the corresponding K_p.

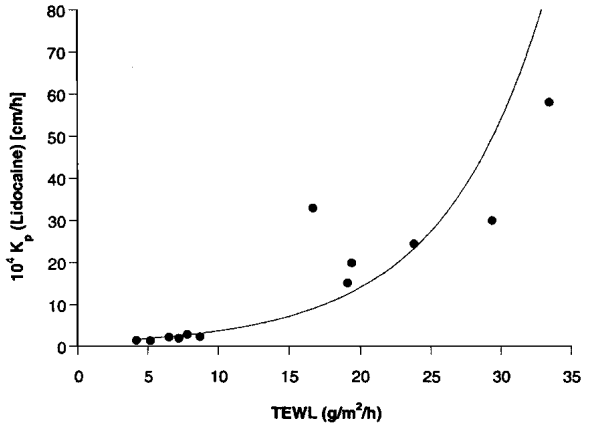

Fig. 5. Passive permeability coefficients (K_p) of lidocaine as a function of skin barrier integrity (measured in terms of TEWL). The empirical exponential fit to the data shown is: $10^4 \cdot K_p = 0.97 \cdot \exp[0.13 \cdot \text{TEWL}]$, with $r^2 = 0.89$.

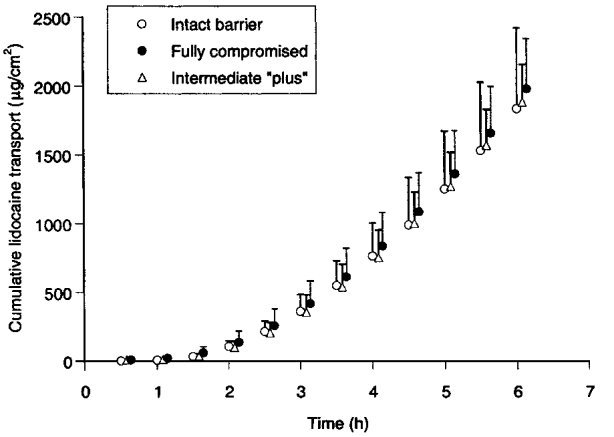

Fig. 7. Iontophoretic transport of lidocaine across skin barriers of different integrities. Each data point represents the mean ± SD (n = 6 for intact and fully compromised barriers; n = 3 for the intermediate "plus" skin).

Subsequently, with K_p known, the concentrations of drug to be administered, and over what surface area, can be calculated so to provide a specific input rate (in terms, for example, of microgram per hour) into the patient.

Though the preceding discussion outlines a method by which the rational transdermal dosing of premature infants may be achieved, the published data on lidocaine transport across infant skin reveals a real concern. Specifically, despite the consistent overall trends in the data observed, there is a significant variability in the results. For drugs of narrow therapeutic indices, therefore, the predicted delivery rates may not result in the desired pharmacodynamic outcome. It was for this reason, then, that we subsequently examined, across porcine skin barriers of differential efficiency (and prepared identically to those used in the passive experiments), the iontophoretic delivery of lidocaine.

The electrotransport results are summarized in Fig. 7, which should be visually contrasted to the passive data in Fig. 1; the two sets of experiments are further compared in Table II. Several important observations can be made. First, as hypothesized in the "Introduction," iontophoresis permits exceptional control of drug delivery (with very modest variability), which is independent of the barrier functionality of the membrane used. This observation is consistent with a previous study (28) that showed that the transdermal delivery of nalbuphine and its prodrugs, across various skin membranes with and without SC, was precisely modulated by iontophoresis. Second, as a corollary to this first point, the estimated transport number of the drug across the skin membranes of different efficiencies is essentially constant (see Table II). Third, much higher levels of drug delivery can be achieved more quickly with iontophoresis compared to passive diffusion. As indicated in Table II, 6 h of electrotransport enables nearly 2 mg of drug to be delivered per cm^2; in contrast, the corresponding passive deliveries of lidocaine across fully functional and completely deranged SC are only on the order of 1 and 100 $\mu g/cm^2$, respectively. It follows, therefore, that

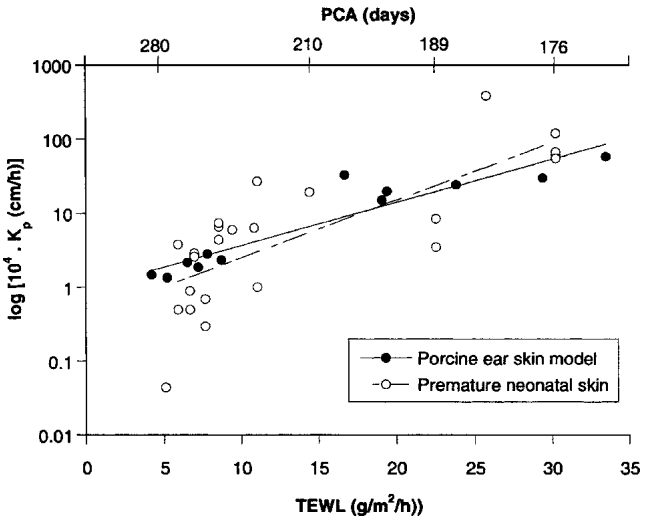

Fig. 6. Comparison between (a) the permeability coefficients (K_p) of lidocaine across porcine skin *in vitro*, as a function of barrier competency (expressed in terms of TEWL), and (b) the K_p of the drug across premature neonatal infant skin, as a function of the "donor's" postconceptional age (PCA). The correspondence between PCA and TEWL was made via Eq. 1 (see text for details).

Table II. Cumulative Lidocaine Delivery (Mean ± SD) After 6 h of Passive and Iontophoretic Transport Across Porcine Skin *in Vitro,* as a Function of Barrier Integrity (the Latter Characterized by the Value of TEWL Relative to That of Intact, Unstripped Skin)

Barrier integrity (relative TEWL)	Lidocaine delivery ($\mu g/cm^2$)		
	Passive[a]	Iontophoresis[b]	t_{D+}[c]
Intact (1.0)	0.7 ± 0.4	1837 ± 583[d]	0.10
Intermediate "plus" (2.0–2.7)	6.9 ± 0.6	1883 ± 275	0.10
Intermediate "less" (2.9–4.2)	66 ± 24	N.D.[e]	—
Fully compromised (4.5–8)	116 ± 69	1979 ± 364[d]	0.11

TEWH, transepidermal water loss.

[a] ANOVA ($p < 0.05$) reveals significant differences between these values.

[b] ANOVA ($p > 0.05$) indicates that these values are statistically indistinguishable.

[c] Lidocaine transport number calculated from the drug flux and applied current in the iontophoretic experiments (29).

[d] n = 6; for all other experiments, n = 3.

[e] N.D. = not determined.

even for skin with effectively no barrier function, the contribution of passive diffusion to the total delivery by iontophoresis is negligible. Equally, recent results from our laboratory (29) demonstrate, for the iontophoretic conditions used, that lidocaine electrotransport is principally (>90%) electromigrative, with little contribution from electroosmosis. Given, furthermore, that the experiments reported here involved electrode and receptor solutions at lower pH than those used in the earlier work (29), the convective solvent flow contribution would be even lower than that measured before. Indeed, the pH values of the electrode and receptor solutions were such that the skin membrane would have been close to its isoelectric point (30), at which essentially no electroosmosis would occur. There is, therefore, a considerable margin of opportunity available over which the iontophoretically delivered amount of lidocaine may be manipulated by adjusting the current. In particular, efficient delivery, which is well-controlled, should be possible with lower levels of current application, minimizing thereby any sensation detectable at the surface of the patient's skin. The efficiency of delivery also means that the area of contact between the active portion of an iontophoretic system and the neonate's skin can probably be kept to a reasonably small value.

Parenthetically, an additional practical advantage should also be highlighted here [and has been identified elsewhere when iontophoresis of drug across deranged skin has been studied (28)]. The proportionality between current dose and amount of drug delivered emphasizes why commercially developed iontophoresis devices will be constant-current or current-modulable systems. In this situation, in accord with Ohm's law, if the resistance of the skin changes, then (as current is fixed) the voltage provided by the device will adjust. In our experiments, because SC stripping decreased skin resistance, then a decreased voltage was required to deliver the same iontophoretic current, which, in turn, meant that drug flux also remained constant across the different membranes. Given that the resistance of premature skin is less than that of the mature barrier (31), it follows that the power requirements of an iontophoresis system for use in premature neonates will be very modest.

In conclusion, we submit that this work demonstrates the feasibility of transdermal drug delivery to premature neonates and reinforces the value of the *in vitro* model used as a means to predict the dosing regimen necessary for an infant as a function of its postconceptional age. Because of the impaired skin barrier function of this patient population, passive transdermal administration is possible but requires continual monitoring and adjustment as the SC evolves to its complete form. An important caveat, though, is that the variability typically seen in percutaneous permeability limits the approach to drugs of wider therapeutic windows, where problems of over- or under-dosing are of less concern. However, iontophoresis can be used for ionizable drugs of appropriate properties to control precisely input across the skin, with markedly less variability, and regardless of the efficiency of the skin barrier. Dose modulation is easily achievable by adjusting the current. Here, lidocaine has been used as a model drug to demonstrate the utility of iontophoresis. As it moves across the skin, under the influence of the electric field, almost exclusively by electromigration, lidocaine is an ideal candidate for iontophoretic delivery. Nevertheless, it is now important to examine the limitations of the approach and to study the delivery of other drugs by eletrotransport, and these objectives form the basis of subsequent work.

ACKNOWLEDGMENTS

This work was partially supported by the Fonds national Suisse de la recherche scientifique.

REFERENCES

1. N. Rutter. The immature skin. *Eur. J. Pediatr.* **155**:S18–S20 (1996).
2. Y. N. Kalia, L. B. Nonato, C. H. Lund, and R. H. Guy. Development of skin barrier function in premature infants. *J. Invest. Dermatol.* **111**:320–326 (1998).
3. M. L. Williams and V. T. Le K. The permeability barrier in the preterm infant. *Eur. J. Pediatr. Dermatol.* **8**:101–106 (1998).
4. D. A. Barrett and N. Rutter. Transdermal delivery and the premature neonate. *Crit. Rev. Ther. Drug Carrier Syst.* **11**:1–30 (1994).
5. N. J. Evans and N. Rutter. Transdermal drug delivery to the newborn infant. In J. Hadgraft and R. H. Guy (eds.), *Transdermal Drug Delivery*, Marcel Dekker, New York, 1989, pp. 155–176.
6. J. B. Besunder, M. D. Reed, and J. L. Blumer. Principles of drug biodisposition in the neonate: a critical evalutation of the pharmacokinetic-pharmacodynamic interface (Part I). *Clin. Pharmacokinet.* **14**:189–216 (1988).
7. N. Barker, J. Hadgraft, and N. Rutter. Skin permeability in the newborn. *J. Invest. Dermatol.* **88**:409–411 (1987).
8. N. J. Evans, N. Rutter, J. Hadgraft, and G. Parr. Percutaneous administration of theophylline in the preterm infant. *J. Pediatr.* **107**:307–311 (1985).
9. R. G. Cartwright, P. H. Cartlidge, N. Rutter, C. D. Melia, and S. S. Davis. Transdermal delivery of theophylline to premature infants using a hydrogel disc system. *Br. J. Clin. Pharmacol.* **29**: 553–559 (1990).
10. M. Amato, M. Isenschmid, and P. Huppi. Percutaneous caffeine application in the treatment of neonatal apnoea. *Eur. J. Pediatr.* **150**:592–594 (1991).
11. N. J. Evans and N. Rutter. Development of the epidermis in the newborn. *Biol. Neonate* **49**:74–80 (1986).
12. L. B. Nonato. *Evolution of Skin Barrier in Premature Neonates,* Ph.D Thesis, University of California, Berkeley, 1997.
13. N. Sekkat and R. H. Guy. Biological models to study skin permeation. In B. Testa, H. Van de Waterbeemd, G. Folkers, and R.H. Guy (eds.), *Pharmacokinetic Optimization in Drug Research*, Wiley-VCH and VHCA, Zürich, 2001, pp. 155–172.
14. N. Sekkat, Y. N. Kalia, and R. H. Guy. Development of an in vitro model for premature neonatal skin: biophysical characterization using transepidermal water loss. *J. Pharm. Sci.* (2003).
15. F. P. Bonina, L. Montenegro, G. Micali, D. P. West, P. Palicharla, and R. L. Koch. In vitro percutaneous absorption evaluation of phenobarbital through hairless mouse, adult and premature human skin. *Int. J. Pharm.* **98**:93–99 (1993).
16. P. G. Green, M. Flanagan, B. Shroot, and R. H. Guy. Iontophoretic drug delivery. In K. A. Walters and J. Hadgraft (eds.), *Pharmaceutical Skin Penetration Enhancement*, Marcel Dekker, New York, 1993, pp. 311–333.
17. P. M. Lai and M. S. Roberts. Iontophoresis. In M. S. Roberts and K. A. Walters (eds.), *Dermal Absorption and Toxicity Assessment*, Marcel Dekker, New York, 1998, pp. 371–414.
18. O. Wong. Iontophoresis: fundamentals. In D. S. Hsieh (ed), *Drug Permeation Enhancement, Theory and Applications*, Marcel Dekker, New York, 1994, pp. 219–246.
19. N. Kanikkannan, J. Singh, and P. Ramarao. In vitro transdermal iontophoretic transport of timolol maleate: effect of age and species. *J. Control. Rel.* **71**:99–105 (2001).
20. D. A. Barrett and N. Rutter. Percutaneous lignocaine absorption in newborn infants. *Arch. Dis. Child.* **71**:F122–F124 (1994).
21. P. Glikfeld, C. Cullander, R. S. Hinz, and R. H. Guy. A new system for in vitro studies of iontophoresis. *Pharm. Res.* **5**:443–446 (1988).
22. A. Naik. *Azidoprofen as a Soft Anti-Inflammatory Agent for the*

Topical Treatment of Psoriasis. Ph.D Thesis, University of Aston, Birmingham, 1990.
23. G. K. Herkes, G. E. McKinnon, and M. J. Eadie. Simultaneous quantitation of salivary carbamazepine, carbamazepine-10,11-epoxide, phenytoin and phenobarbitone by high-performance liquid chromatography. *J. Chromatogr.* **496**:147–154 (1989).
24. D. R. Friend. In vitro skin permeation techniques. *J. Control. Rel.* **18**:235–248 (1992).
25. W. R. Ravis. Data interpretation and analysis in percutaneous absorption studies. In B.W. Kemppainen and W. G. Reifenrath (eds.), *Methods for Skin Absorption*, CRC Press, Boca Raton, 1990, pp. 147–163.
26. D. A. Barrett, N. Rutter, and S. S. Davis. An in vitro study of diamorphine permeation through premature human neonatal skin. *Pharm. Res.* **10**:583–587 (1993).
27. D. A. Barrett and N. Rutter. Percutaneous lignocaine absorption in newborn infants. *Arch. Dis. Child.* **71**:F122–F124 (1994).
28. K. C. Sung, J. Y. Fang, and O. Y. Hu. Delivery of nalbuphine and its prodrugs across skin by passive diffusion and iontophoresis. *J. Control. Rel.* **67**:1–8 (2000).
29. D. Marro, Y. N. Kalia, M. B. Delgado-Charro, and R. H. Guy. Contribution of electromigration and electro-osmosis to iontophoretic drug delivery: I. Lidocaine hydrochloride. *Pharm. Res.* **18**:1707–1713 (2001).
30. D. Marro, R. H. Guy, and M. B. Delgado-Charro. Characterization of the iontophoretic permselectivity properties of human and pig skin. *J. Controlled Release* **70**:213–217 (2001).
31. Y. N. Kalia, L. B. Nonato, C. H. Lund, and R. H. Guy. Development of skin barrier function in premature infants. *J. Invest. Dermatol.* **111**:320–326 (1998).