International Journal of Pharmaceutics 435 (2012) 76–82

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Passive and iontophoretic transdermal delivery of phenobarbital: Implications in paediatric therapy

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ARTICLE INFO

Article history:
Received 2 December 2011
Received in revised form 14 February 2012
Accepted 15 February 2012
Available online 23 February 2012

Keywords:
Paediatric
Transdermal
Iontophoresis
Phenobarbital
Premature
Tape-stripping

ABSTRACT

The objective of this investigation was to evaluate phenobarbital transdermal delivery for possible use in paediatric care. *In vitro* experiments were performed using intact pig skin and barriers from which the stratum corneum had been stripped to different extents to model the less resistant skin of premature babies. Cathodal iontophoretic delivery of phenobarbital was superior to anodal transport and optimised delivery conditions were achieved by reduction of competing co-ion presence in the drug formulation. Phenobarbital transport across intact or partially compromised skin was controlled by iontophoresis which was more efficient than passive diffusion. Across highly compromised skin, however, passive diffusion increased drastically and iontophoretic control was lost. Overall, this study demonstrates the feasibility of phenobarbital transdermal delivery for paediatric patients.

1. Introduction

Phenobarbital is a barbiturate drug used in the treatment of different forms of paediatric epilepsy and status epilepticus (BNF for children, 2011) being the first-line choice to control neonatal seizures (Lehr et al., 2005; Blume et al., 2009; Ouvrier et al., 1982). It is also used to treat neonatal abstinence syndrome both in the case of sedative-hypnotic withdrawal and as an adjunct therapy to treat opiate withdrawal symptoms (Bio et al., 2011; Finnegan and Kandall, 2005; Osborn et al., 2010). Due to developmental changes in children, the pharmacokinetics of phenobarbital are highly variable in this population; doses are, therefore, titrated according to the individual's response for adequate seizure control while avoiding, at the same time, adverse effects due to supra-optimal levels (BNF for children, 2011; Finnegan and Kandall, 2005; Heimann and Gladtke, 1977; Lehr et al., 2005; Touw et al., 2000). The target therapeutic plasma concentrations are set between 40 and 180 μmol mL^{-1} depending on the application envisaged (Bio et al., 2011; Finnegan and Kandall, 2005; Lehr et al., 2005; Touw et al., 2000). Heimann and Gladtke (1977) found that the elimination half-life of phenobarbital in mature neonates (118 ± 16 h) is significantly longer than that in infants of 2–12 months (63 ± 5 h) and in children aged 2–5 years (68 ± 3 h). Touw et al. (2000) found that the elimination half-life varied between 48 and 147 h for a group of 19 term and preterm neonates. Phenobarbital clearance is slowest for newborns, increasing rapidly during the first two weeks of life and reaching a peak at 6–12 months. On the other hand, the total clearance normalized per kg body weight appears to decrease with increasing maturity (Touw et al., 2000; Lehr et al., 2005). The average clearance of phenobarbital in neonates is about 4.3 mL h^{-1} kg^{-1} (Touw et al., 2000) whereas, for older children, a mean value of approximately 8 mL h^{-1} kg^{-1} is observed (Botha et al., 1995; Heimann and Gladtke, 1977; Winter, 2010). Phenobarbital distribution volume decreases with age and with gestational age; Touw et al. (2000) reported a mean volume of distribution of 0.71 ± 0.21 L kg^{-1} for term and preterm neonates. Heimann and Gladtke (1977) used a two-compartmental model to describe phenobarbital kinetics in children of several age groups, from term neonates up to 5 years old, and found a modest age dependency for both the volume of distribution in the steady-state and the volume of the central compartment; the values reported for V_{ss} ranged from 0.85 ± 0.06 to 0.67 ± 0.07 L kg^{-1}.

Phenobarbital is well absorbed orally (Winter, 2010); however, tablets are not suitable for neonates and young children and the only approved oral alternative described in the BNF (BNF for children, 2011) contains 38% ethanol. Regular administration of this elixir may cause alcohol toxicity, especially in neonates. As consequence, extemporaneous formulations, including suspensions from crushed tablets, are sometimes prepared (Cober and Johnson,

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doi:10.1016/j.ijpharm.2012.02.026

2007; Colquhoun-Flannery and Wheeler, 1992). Slow intravenous injections are also frequently used, but care must be taken to dilute the parenteral formulation (200 mg mL^{-1} phenobarbital in 90% propylene glycol) to provide suitable doses for young infants and children and to avoid accumulation of the cosolvent (Allagaert et al., 2010; BNF for children, 2011).

Passive transdermal delivery of phenobarbital has been suggested as an alternative route of administration (Bonina et al., 1993). In this earlier *in vitro* study, drug flux across excised skin from premature infants (29–35 weeks gestational age) was approximately 4-fold greater (0.4 ± 0.13 μg h^{-1} cm^{-2}) than that (0.1 ± 0.02 μg h^{-1} cm^{-2}) through either adult or full-term (37–40 weeks gestational age) skin. In general, fluxes were inversely related to the gestational age of the donor. It was estimated that a 25 cm^2 transdermal patch would be sufficient to provide an average steady-state plasma concentration of 3.2 mg L^{-1} (14 μM) for a 1 kg neonate. However, the feasibility of the approach employed may be questioned as the vehicle used in this work was pure ethanol which is known to be toxic to preterm newborns when absorbed across the skin from cleaning products (Harpin and Rutter, 1982). Further, the total skin surface area of a neonate varies between 0.03 and 0.25 m^2 depending on gestational age (Touw et al., 2000). While there are no guidelines concerning the maximum acceptable size of a transdermal patch for neonatal use, values in the range of 1–10 cm^2 would be consistent with current usage in adults: for these individuals, total skin area is ~2 m^2, while largest patches in use are in the order of 50 cm^2. Finally, the systemic concentration achievable (14 μM) is lower than the recommended target for paediatric patients (40–180 μM).

Here, an optimised approach to the delivery of phenobarbital across both intact and premature skin is proposed. Using iontophoresis as a physical enhancement method, improved delivery rates of the drug are achieved compared to passive diffusion suggesting that smaller patch sizes can be used to attain therapeutic systemic levels. With iontophoresis, the much more water-soluble sodium salt of phenobarbital is preferred eliminating the need for incorporation of co-solvents (such as ethanol) in the topical formulation. The aqueous solubility of sodium phenobarbital (molecular weight = 254.2 Da) is 1 g/mL, approximately 1000-fold greater than that of the free acid. Intact and premature neonatal skin barriers were modelled *in vitro* using pig skin, from which stratum-corneum was differentially tape-stripped as previously described (Sekkat et al., 2004a,b).

2. Materials and methods

2.1. Chemicals

Sodium phenobarbital (PHEN), silver wire (99.99%), silver chloride (99.999%), sodium hydroxide pellets, and NaOH 50% solution (ion chromatography eluent grade) were purchased from Sigma–Aldrich (Gillingham, UK). Potassium dihydrogen phosphate, HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) and sodium chloride were obtained from Acros (Geel, Belgium). Acetonitrile and hydrochloric acid were provided by Fisher Scientific (Loughborough, UK). All reagents were at least analytical grade and deionised water (resistivity ≥ 18.2 MΩ cm, Barnsted Nanopure Diamond™, Dubuque, IA) was used for the preparation of all solutions.

2.2. Skin

Fresh pig skin was obtained from a local slaughterhouse, cleaned under cold running water, and stored in a refrigerator until the following day. Abdominal skin was dermatomed (Zimmer™ Electric Dermatome, Dover, OH) to a nominal thickness of 750 μm, cut into (10 cm × 10 cm) pieces, wrapped individually in Parafilm™, and then kept in a freezer (−20 °C) until use. Prior to the experiments, the skin was thawed at room temperature for 30 min and excess hair was cut with scissors. The large piece of skin was then cut into 4 portions. One served as representative of the intact skin barriers while the others were subjected a tape-stripping procedure to create different degrees of compromised skin. The intact barriers were characterised by transepidermal water loss (TEWL) measurements (AquaFlux AF-102, Biox Systems Ltd., London, UK) of 9.0 ± 1.7 g m^{-2} h^{-1}. The three other pieces of skin were repeatedly tape-stripped (2 cm × 2 cm, Scotch Book Tape, 3 M, St. Paul, MN) to progressively remove the stratum corneum. A 1.5 cm × 1.5 cm template was affixed onto the skin before the stripping procedure started to ensure the removal of stratum corneum was from the same location. Periodic measurement of TEWL allowed the degree of barrier compromise to be quantified and full barrier impairment was defined when the removal of three consecutive tape strips did not alter TEWL. The number of tape strips required to produce a fully compromised barrier varied between 13 and 23. The second and third pieces of skin were tape-stripped until TEWL reached values of between 20–40% and 60–80%, respectively, of the TEWL recorded for the fully compromised barrier. Thus, three levels of barrier function impairment were studied: 20–40% (intermediate "less" barrier), 60–80% (intermediate "plus" barrier), and 100% (fully compromised barrier).

2.3. Iontophoresis set-up

Side-by-side two-compartment diffusion cells (active transport area = 0.78 cm^2, volume = 3.3 mL) were used in all experiments. The skin was mounted between the two chambers with the epidermal side oriented towards the cathode compartment. The receptor chamber always held 154 mM sodium chloride solution (unbuffered, pH ~6). Prior to the start of the transport study, the skin was left for 30 min in contact with the donor vehicle without drug, and 154 mM sodium chloride in the receptor chamber. The compartments were then emptied and refilled with a donor solution containing sodium phenobarbital and with fresh receptor solution. Both compartments were magnetically stirred (Multipoint-6 Stirrer, Thermo Scientific Variomag, Cole-Parmer, UK) throughout the experiment. A direct constant current of 0.4 mA (0.5 mA cm^{-2}) was delivered using Ag/AgCl electrodes and a power supply (KEPCO 1000 M, Flushing, NY, USA). Hourly samples (0.5 mL) of the receptor phase were withdrawn for analysis and replaced with fresh receptor solution. Experiments, which compared phenobarbital iontophoretic delivery through different skin barriers, also monitored passive permeation (same donor solution) post-current termination. Separate passive diffusion controls (no current) through both intact and compromised skin were also performed. The details of the experiments performed are in Table 1.

Another series of experiments examined the effect of iontophoresis on the passive permeability of intact skin to phenobarbital. Prior to the permeation study, and in the absence of phenobarbital (*i.e.*, with a donor compartment containing water, pH 8.5, and receptor compartment containing unbuffered 154 mM NaCl), a 0.4 mA current was applied for 5 h. At this point, the current was terminated and the compartments were then emptied and refilled with a donor solution containing phenobarbital and with fresh receptor solution. Passive diffusion was then followed for 24 h. To account for any effect of skin hydration during the pre-iontophoresis, the same experiment was repeated without application of current and with the donor and receptor compartments being filled with water and 154 mM NaCl, respectively.

Table 1
Experiments performed to characterise phenobarbital transdermal delivery through intact, pre-treated and impaired skin.

Skin barrier	Donor	PHEN (mM)	Experiment settings and duration	*n*
Intact	Water (pH 8.5)	50	(1) Cathodal iontophoresis (5 h), then passive diffusion (5 h)	3
			Passive diffusion (24 h)	3
	50 mM NaCl (pH 7.4)	15	(2) Cathodal iontophoresis (5 h)	4
			Anodal iontophoresis (5 h)	4
			Passive diffusion (24 h)	6
	Water (pH 7.4)	15	(3) Cathodal iontophoresis (5 h)	6
			(4) Cathodal iontophoresis (5 h). Donor solution exchanged hourly	5
	10 mM HEPES (pH 7.4)	15	(5) Cathodal iontophoresis (5 h). Donor solution exchanged hourly	5
Pre-treated	Water (pH 8.5)	50	Pre-treatment: 0.4 mA for 5 h followed by passive diffusion (24 h)	4
			Pre-treatment: hydration for 5 hfollowed by passive diffusion (24 h)	
Impaired: 20–40% 60–80% 100%	Water (pH 8.5)	50	Cathodal iontophoresis (5 h), then passive diffusion (5 h)	3–5
			Passive diffusion (24 h)	3–5

2.4. Phenobarbital and chloride analysis

Quantification of phenobarbital was performed by high performance liquid chromatography with UV detection (215 nm). The method was modified from a previous publication (Sekkat et al., 2004b) and used a Jasco HPLC system (PU-980 pump with an AS-1595 autosampler, a UV-975 UV-VIS detector, and an Acclaim 120, C18 (150 mm × 4.6 mm, 5 μm) reversed-phase column (Dionex, UK) which was thermostated at 30 °C. The mobile phase, pumped at 1 mL min^{-1}, consisted of phosphate buffer (0.067 M KH_2PO_4) and acetonitrile (70:30) and the pH was adjusted to 6 with NaOH.

Chloride was analysed by ion chromatography with suppressed conductivity detection (Sylvestre et al., 2008a,b) using a Dionex system (Sunnyvale, CA) comprising a GP-50 gradient pump, an AS-50 autosampler and thermal compartment, and an ED-50 electrochemical detector. The mobile phase, 35 mM NaOH, was pumped isocratically (1 mL min^{-1} flow rate) through a Dionex IonPacTM AS16 (250 mm × 4 mm) column thermostated at 30 °C and connected to a Dionex ASRS Ultra II suppressor (4 mm) set at a current of 90 mA.

2.5. Data analysis and statistics

Data analysis was performed using Graph Pad Prism V.5.00 (Graph Pad Software Inc., CA, USA). Unless otherwise stated, data are presented as the mean ± standard deviation. Transport fluxes were calculated as the amounts delivered during a permeation period divided by the length of that period. Statistical significance was set at $p < 0.05$. Comparisons made between different sets of data were assessed by either a two-tailed unpaired *t*-test (for 2 groups) or a one-way ANOVA (for >2 groups) followed by Tukey's post-test. Comparison of fluxes at different times was assessed by repeated-measures ANOVA followed by Tukey's post-test.

The corrected transference number ($t_{COR,PHEN}$) of phenobarbital was computed according to Faraday's law (Phipps and Gyory, 1992):

$$t_{PHEN} = \frac{J_{COR,PHEN} \cdot z \cdot F}{I} \quad (1)$$

where, $J_{COR,PHEN}$ is the corrected flux, I is the current intensity applied, F is Faraday's constant, and z the absolute value of the drug valence. Transference numbers were calculated from the corrected fluxes representing those observed after 5 h iontophoresis (J_{PHEN}) minus the passive diffusion rate 5 h post-current termination.

3. Results and discussion

3.1. Passive diffusion across intact skin

The passive diffusion of phenobarbital from a 50 mM aqueous drug solution (pH 8.5) across (i) untreated skin, (ii) skin pre-treated with 0.4 mA current for 5 h, and (iii) skin hydrated for 5 h is shown in Fig. 1 in terms of drug permeation as a function of time. The cumulative amounts transported (±SD) 24 h post-drug application were: 91.7 ± 33.7, 415 ± 125, and 222 ± 35.5 nmol cm^{-2} for untreated, pre-iontophoresed, and pre-hydrated skin, respectively. Passive diffusion across pre-iontophoresed skin was significantly higher than through untreated ($p < 0.01$) and pre-hydrated ($p < 0.05$) skin. Increased permeability of skin previously exposed to direct current has been previously reported (Green et al., 1992).

3.2. Iontophoretic delivery across intact skin

The first donor solution tested was 50 mM sodium phenobarbital in water (pH 8.5) where the drug (pK_a = 7.3) (The Merck Index, 2006) was ~93% ionised. Cathodal iontophoresis resulted in drug delivery that was 385-fold higher than passive diffusion (Fig. 2). After only 1 h of current application, the phenobarbital flux was 222 ± 94.4 nmol h^{-1} increasing to 387 ± 57.2 nmol h^{-1} by 3 h. At the end of the experiment (5 h), the flux was 341 ± 64.5 nmol h^{-1}.

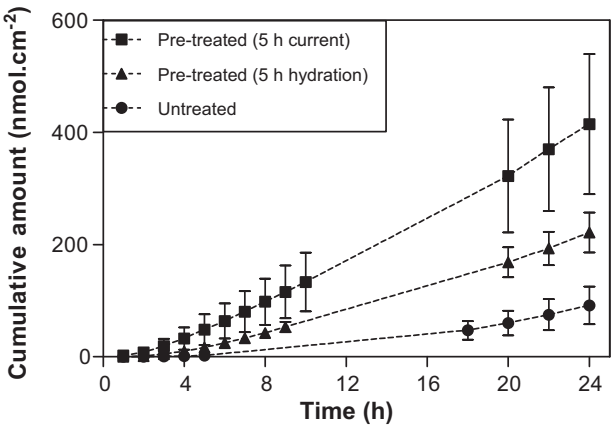

Fig. 1. Passive diffusion of phenobarbital through intact pig skin. Pre-treatment either involved 5 h direct current at 0.4 mA or 5 h hydration without current. Phenobarbital was not present in the pre-treatment periods. Data are represented as the mean ± SD.

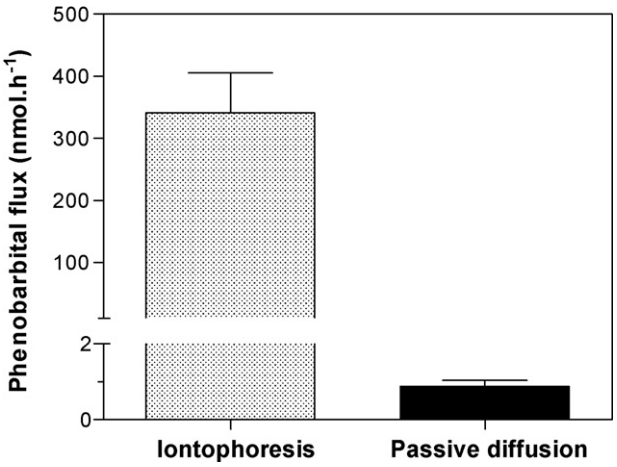

Fig. 2. Passive and iontophoretic transdermal fluxes (mean ± SD) of phenobarbital after 5 h from a 50 mM drug solution.

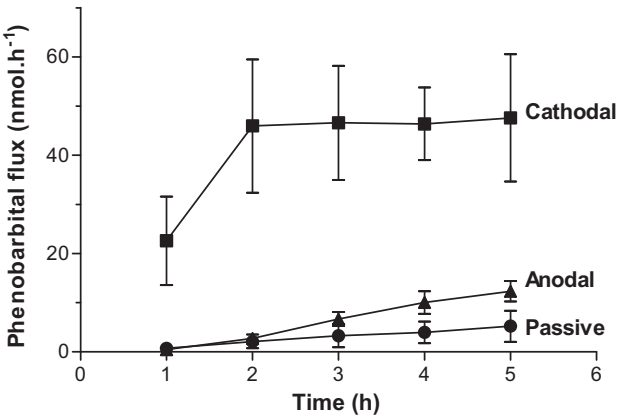

Fig. 3. Anodal and cathodal iontophoresis of phenobarbital when delivered from a 15 mM drug solution at pH 7.4. The passive diffusion control is also shown. Data points are represented as mean ± SD.

A second series of iontophoresis experiments compared anodal *versus* cathodal delivery of phenobarbital at pH 7.4 where phenobarbital exists in essentially equal concentrations of the ionised and unionised forms. The former, of course, can be delivered from the cathode by electro-repulsion, while the latter may be transported from the anode by electro-osmosis. A donor concentration of 15 mM was used because of phenobarbital's lower solubility at this pH. Chloride ions are required to ensure adequate electrochemistry at the anode, and 50 mM NaCl was therefore added to both the cathodal and anodal solutions. Cathodal iontophoresis (Fig. 3) was much more efficient than anodal. When the contribution of passive diffusion was taken into account, the corrected cathodal and anodal fluxes at 5 h were 42.4 ± 13.3 and 7.1 ± 3.8 nmol h^{-1}, respectively. These results are in good agreement with previous data (*e.g.*, for 5-fluorouracil (Merino et al., 1999) and for phenytoin (Leboulanger et al., 2004)) which showed that electromigration is a much more efficient transport mechanism than electroosmosis. The reduced cathodal delivery observed here relative to that observed at pH 8.5 is explained by the lower concentration of ionised drug employed, decreasing from 46.3 mM (~93% of 50 mM) to 7.5 mM (~50% of 15 mM) and by the presence of 50 mM competing chloride ions in the pH 7.4 experiment.

The effect of co-ion competition on cathodal delivery by electro-repulsion was then investigated. In an initial experiment, a 15 mM PHEN solution, the pH of which had been adjusted to 7.4 with HCl was used, resulting in introduction of Cl^- at a concentration of approximately 6 mM. As this represents a lower level of chloride ions compared to the earlier experiment, an increase in t_{PHEN} and PHEN flux might have been expected. However, the concentration of competing chloride increases above the initial 6 mM during the experiment because of the gradual release of Cl^- from the cathode as the electrochemistry reduces AgCl to Ag (Fig. 4). This implies that transport number of the drug would decrease as the experiment proceeds.

A subsequent experiment attempted to mitigate the impact of chloride accumulation on the cathodal flux of PHEN by refreshing the donor solution every hour. In a related experiment, 10 mM HEPES was employed as a buffer for the 15 mM sodium phenobarbital donor solution. This provided good buffer capacity without requiring any adjustment to pH 7.4 with HCl (thereby avoiding the introduction of extra chloride ions). Again, to counter the build-up of chloride ions released from the electrode, the donor solution was refreshed hourly. It was calculated that Cl^- release from the cathode would contribute (in the diffusion cells used) a concentration of 4.5 mM for every hour of 0.4 mA current applied. Fig. 4 illustrates the anticipated evolution of chloride concentration in the donor solution as a function of time under the experimental conditions employed. The predicted values agree closely with those measured experimentally by ion chromatography.

The PHEN fluxes were inversely related to the Cl^- concentration present in the donor solution after 5 h of current application. The greater the co-ion competition with PHEN, the lower the drug transport (Fig. 4). Chloride accumulation in the cathodal chamber plays a key role in the iontophoretic delivery of negatively charged

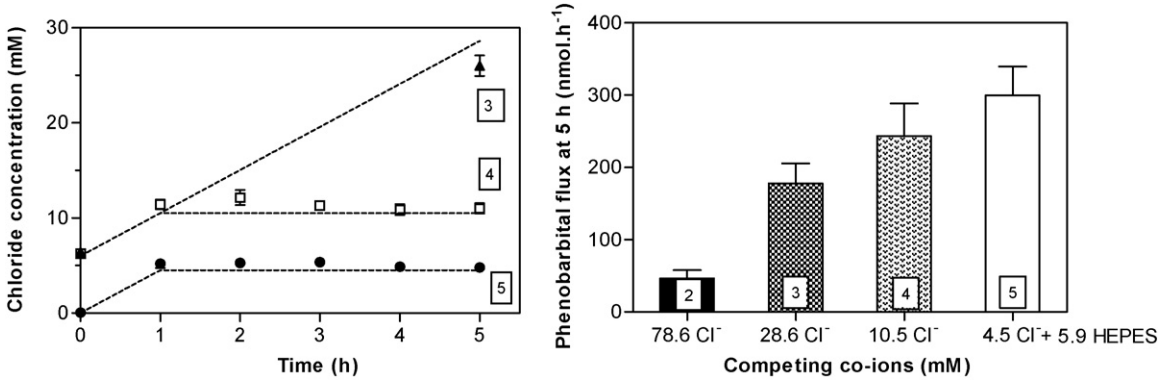

Fig. 4. *Left panel:* estimated donor concentration of chloride ions (dashed lines) and the corresponding measurements (symbols, mean ± SD) for experiments 3 (▲), 4 (□), and 5 (●) as indicated in Table 1. *Right panel:* cathodal delivery of phenobarbital from different donor solutions (pH 7.4) containing various amounts of competing co-ions (experiments 2–5 in Table 1). The flux values (mean ± SD) were determined after 5 h of iontophoresis (0.4 mA).

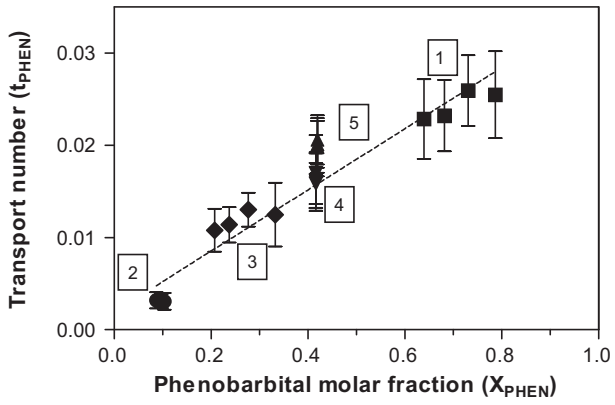

Fig. 5. Transport number of phenobarbital (t_{PHEN}, mean ± SD) as a function of molar fraction (X_{PHEN}). Values of the latter parameter were calculated from the concentrations of phenobarbital, HEPES, and chloride. The sources of Cl^- included NaCl (used as background electrolyte), HCl (used to adjust the donor solution pH), and the electrode electrochemical reaction. Data expressed by the same symbol and number represent the experimental condition as identified in Table 1. The dashed line is the linear regression through all data points except those obtained with HEPES: $t_{PHEN} = 0.002\ (\pm 0.001) + 0.033\ (\pm 0.002)\ X_{PHEN}$ ($r^2 > 0.8$).

drugs as previously demonstrated for dexamethasone phosphate (Sylvestre et al., 2008a,b) and needs careful optimisation.

The transport number of phenobarbital (t_{PHEN}) was linearly proportional ($r^2 \approx 0.80$) to the drug's molar fraction in the vehicle (Fig. 5). Following the principles demonstrated by Mudry et al. (2006) for cation electrotransport, and assuming their validity for the anionic PHEN, the maximum transport number of the drug at pH 7.4, *i.e.*, in the absence of competing co-ions, is estimated to be 0.035 (determined by substitution of $X_{PHEN} = 1$ in the linear regression equation given in Fig. 5).

3.3. Permeation across compromised skin

The objective of this part of study was to examine the permeation of phenobarbital across barriers representative of those found in premature neonates whose stratum corneum may be absent or not fully developed. Three impaired levels of barrier function were evaluated against intact skin for passive as well as iontophoretic delivery of the drug. The average TEWL measurements ($g\,m^{-2}\,h^{-1}$) across the different skin barriers were as follows: 11 ± 1, 44 ± 10, 114 ± 20, and 158 ± 25, respectively, for intact, intermediate "less"(20–40%), intermediate "plus" (60–80%), and fully compromised skin. The TEWL values were significantly higher than those reported in previous studies, which validated the usefulness of serially stripped pig skin as a model for that of the developing neonate and subsequently employed the approach to predict the transdermal permeation of phenobarbital, caffeine, and lidocaine (Sekkat et al., 2004a,b). The discrepancy is between a factor of two and four and is probably due to the different TEWL devices employed in the two studies (*i.e.*, the study closed-chamber evaporimeter (AquaFlux AF102, Biox) used here *versus* an open-chamber instrument (EP1, Servomed) used before (Farahmand et al., 2009; Imhof et al., 2009). Other factors may have played a part, such as the number of tape-strips used to remove the stratum corneum, and the pressure with which the tapes were applied (Escobar-Chavez et al., 2008; Rubio et al., 2011).

Passive diffusion of phenobarbital increased dramatically as the stratum corneum was progressively compromised (Fig. 6). The flux at 5 h through intact skin was only $0.9 \pm 0.2\ nmol\,h^{-1}$, but increased to $810 \pm 251\ nmol\,h^{-1}$ for fully compromised skin. Transport through barriers with intermediate levels of impairment levels fell between these two extremes: $31 \pm 12\ nmol\,h^{-1}$ and $561 \pm 179\ nmol\,h^{-1}$ for 20–40% and 60–80% of barrier disruption, respectively.

Previously, a similar phenobarbital permeation rate across intact full-thickness pig ear skin was measured when the drug was delivered from a saturated solution of the unionised drug (4.3 mM) at pH 5 (Sekkat et al., 2004b). Permeation across fully compromised skin was ~30 times higher than that through the intact barrier. In contrast, the enhancement factor observed in this work was more than 900-fold due, at least in part, to the fact that most of the drug was ionised (~3.7 mM unionised) and hence in a less favourable form for passive permeation. With progressive removal of the stratum corneum, the ionised and neutral forms of phenobarbital permeated through the less-resistant skin barrier at much higher rates. Similar behaviour has been seen for 5-fluorouracil (Fang et al., 2004), with removal of the stratum corneum leading to an increase in passive diffusion from <0.03 $\mu mol\,cm^{-2}$ in 6 h to approximately 17 $\mu mol\,cm^{-2}$ a difference of more than 550-fold.

Fig. 6 summarizes the iontophoretic delivery of phenobarbital through compromised skin barriers. The fluxes measured during 5 h of iontophoresis (0.4 mA) application followed by 5 h of passive diffusion are shown. Table 2 presents the fluxes during the last hour of the permeation studies. The iontophoretic fluxes observed increased with the level of skin impairment but complete removal of the stratum corneum only resulted in a 3.6-fold enhancement relative to intact skin (Table 2). When the contribution of passive diffusion is taken into account, the corrected iontophoretic fluxes, and the corresponding t_{PHEN}, are very similar for all skin barriers tested (Table 2). In fact, no significant differences were found between these values. It follows that while iontophoretic flux remained constant and independent of the skin barrier function, passive diffusion increased remarkably as the skin was progressively compromised and eventually overshadowed any benefits from iontophoresis. Qualitatively, these results are consistent with those reported for lidocaine hydrochloride (Sekkat et al., 2004b), for which the total iontophoretic delivery was practically

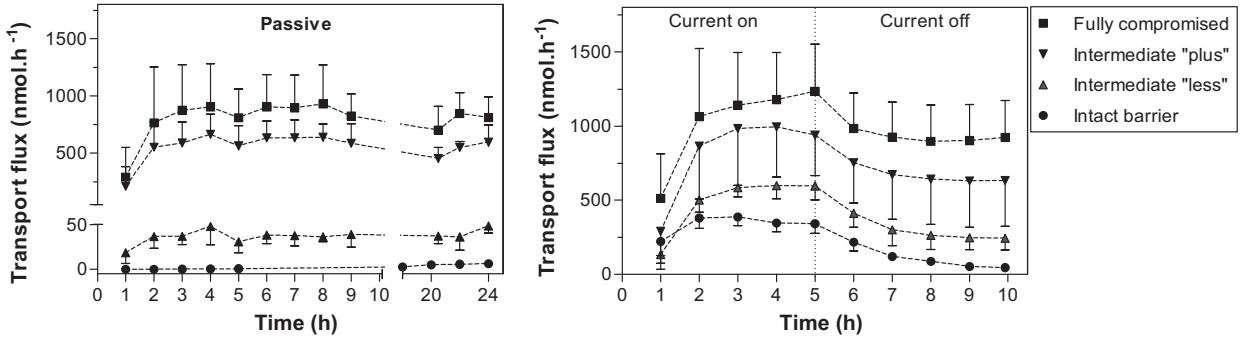

Fig. 6. Passive (left panel) and iontophoretic (right panel) transport (mean ± SD) of phenobarbital delivered from a 50 mM drug solution through intact and compromised skin barriers. The right panel also shows the passive diffusion of phenobarbital post-current termination at 5 h.

Table 2
Fluxes of phenobarbital (μmol h^{-1}, mean ± SD), through differentially impaired skin barriers, after 5 h of iontophoresis, and after a further 5 h of passive diffusion post-current application. The corrected values ($J_{COR,PHEN}$) are the differences between the measurements in the first two columns and are used to calculate the transport number (t_{PHEN}) shown.

Skin barrier	Iontophoresis J_{PHEN}	Passive post-iontophoresis	Corrected $J_{COR,PHEN}$	$t_{PHEN} \times 10^2$
Intact	0.34 ± 0.06	0.05 ± 0.01	0.30 ± 0.07	2.0 ± 0.4
Intermediate "less"	0.60 ± 0.09	0.24 ± 0.08	0.35 ± 0.08	2.4 ± 0.5
Intermediate "plus"	0.94 ± 0.27	0.63 ± 0.31	0.31 ± 0.09	2.1 ± 0.6
Fully compromised	1.23 ± 0.32	0.92 ± 0.25	0.31 ± 0.08	2.1 ± 0.5

the same across intact (1.8 ± 0.5 mg cm^{-2}) and tape-stripped skin (1.9 ± 0.3 mg cm^{-2}). In contrast, the passive permeability of lidocaine HCL increased from $7 \times 10^{-4} \pm 4 \times 10^{-4}$ mg cm^{-2} across intact skin to 0.1 ± 0.07 mg cm^{-2} through a fully tape-stripped barrier. Quantitatively, however, the difference between the behaviour of lidocaine and phenobarbital is important. In the case of lidocaine, the passive diffusion of the drug even across fully compromised skin is still an order of magnitude smaller than iontophoretic delivery; electrotransport can be used, therefore, to control drug input independent of the status of skin barrier function. For phenobarbital, on the other hand, passive transport increases significantly with progressive derangement of the barrier, ultimately overwhelming iontophoretic delivery, which is no longer able to exercise control over the absorption of the drug when the stratum corneum has been compromised.

Finally, a summary of the passive and iontophoretic transport of phenobarbital as a function of TEWL (reflecting barriers of varying competence) is shown in Fig. 7. The slopes of the linear regressions through the passive and iontophoretic fluxes are not significantly different. This emphasizes the point made above that, once the function of the stratum corneum has been undermined (>50%), the passive transport of phenobarbital exceeds that due to iontophoresis and dominates the transdermal delivery of the drug.

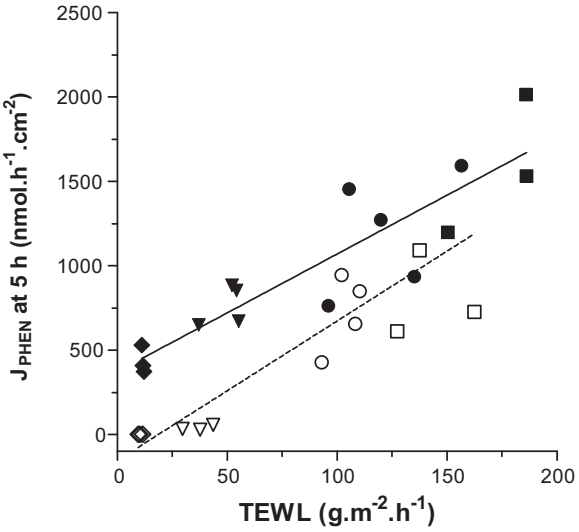

Fig. 7. Total passive and iontophoretic fluxes of phenobarbital as a function of TEWL across skin barriers of different competencies. Open symbols refer to passive diffusion alone; filled symbols reflect the total drug flux when an iontophoretic current is applied. Intact, intermediate "less" (20–40%), intermediate "plus" (60–80%) and fully compromised skin barriers are respectively symbolized by diamonds, triangles, circles, and squares. Linear regressions through the passive and iontophoretic results were: $J_{Passive} = -156\ (\pm 97) + 8.3(\pm 1.1) \times$ TEWL and $J_{ionto} = 372\ (\pm 101) + 7.0(\pm 0.9) \times$ TEWL, with r^2 values of 0.85 and 0.82, respectively.

3.4. Feasibility of phenobarbital transdermal delivery

Phenobarbital is used for different purposes in neonatal and paediatric patients. Examination of the doses used for different indications (BNF for children, 2011; Bio et al., 2011; Finnegan and Kandall, 2005; Osborn et al., 2010) quickly reveals that the transdermal route would not provide the initially large doses required to treat status epilepticus (20 mg kg^{-1} for neonates) or the loading doses required for epilepsy and neonatal abstinence syndrome. However, the maintenance doses for status epilepticus are much lower: 2.5–5 mg kg^{-1} once or twice a day for both neonates and children aged 1 month to 12 years (BNF for children, 2011). For epilepsy, the maintenance doses are 2.5–5 mg kg^{-1} day^{-1} and up to 2.5–8 mg kg^{-1} day^{-1} for neonates and children (1 month to 12 years) respectively (BNF for children, 2011). The maintenance doses recommended for treating neonatal withdrawal symptoms fall in the range 2–10 mg kg^{-1} day^{-1} (Bio et al., 2011; Finnegan and Kandall, 2005; Osborn et al., 2010). The phenobarbital doses required for any of the indications mentioned in children older than 12 years are too large for transdermal administration.

Table 3 calculates the passive and iontophoretic patch sizes required to deliver maintenance doses between 2 and 10 mg kg^{-1} day^{-1} (0.3–1.6 μmol kg^{-1} h^{-1}) *i.e.*, amounts spanning the three potential applications of phenobarbital discussed above. The fluxes used in these calculations were those measured when the donor solution was 50 mM of drug in water at pH 8.5.

Assuming a quantitative *in vitro–in vivo* correlation, transdermal delivery of phenobarbital to neonates (including premature and full-term) appears feasible. Indeed, passive would be sufficient for premature neonates with significantly immature skin. However, as the skin barrier matures, iontophoresis would become progressively more effective in providing desirable rates of delivery while keeping patch size reasonable (Table 3). For safety reasons, and the

Table 3
Estimated patch sizes required to deliver maintenance doses of phenobarbital assuming that the *in vitro* fluxes determined in this work are reflective of those achievable *in vivo*.

Skin type	Intact	Intermediate "less"	Intermediate "plus"	Fully compromised
Passive				
In vitro flux (μmol h^{-1} cm^{-2})[a]	Negligible	0.1 ± 0.02	0.8 ± 0.2	1.1 ± 0.4
Patch size required (cm^2 kg^{-1})		3–16	0.4–2	0.3–1.5
Iontophoresis (0.5 mA cm^{-2})				
In vitro flux (μmol h^{-1} cm^{-2}) [a]	0.5 ± 0.1	0.7 ± 0.1	1.2 ± 0.4	1.5 ± 0.4
Area per electrode (cm^2 kg^{-1})	0.6–3.2	0.4–2.3	0.25–1.3	0.2–1.1
Total patch size (cm^2 kg^{-1}) [b]	1.2–6.4	0.8–4.6	0.5–2.6	0.4–2.2

[a] Fluxes are the average values measured after 2–5 h of iontophoresis (0.5 mA cm^{-2}) or passive diffusion.
[b] Assuming that the areas occupied by the anodal and cathodal electrode formulations are the same.

variable degree of barrier immaturity in the premature neonate, transdermal patches with rate-limiting membranes may be preferable, to ensure rate-control and to avoid potential toxicity. A key challenge with premature neonates is to compensate the rate of drug delivery for the degree of barrier maturation.

Premature neonates of only 23–25 weeks gestational age may require more than 4 weeks to develop a full functional stratum corneum (Kalia et al., 1998); whereas those born at 32 weeks of more have a barrier function that is close to fully functional. To treat this "moving target" would require passive patches in a variety of sizes (and even designs, *e.g.*, rate-controlling *versus* matrix); alternatively iontophoresis might prove more useful in that the intensity and duration of current can be "tuned" to provide the required drug input. This flexibility in dosage form design and operation would rely, of course, on the application of TEWL measurements to pinpoint barrier function status in the patient (Fluhr et al., 2006; Levin and Maibach, 2005).

Table 3 also shows that iontophoresis may deliver therapeutic amounts of phenobarbital to infants of 1 month or more to young children. For older children, however, phenobarbital transdermal delivery may not be useful because the requisite patch area becomes too large. For example, the patch sizes required to deliver 2–10 mg kg^{-1} day^{-1} would be 4–20 cm^2, 6–32 cm^2, and 12–64 cm^2 for paediatric patients weighing 3, 5 and a 10 kg, respectively.

Ultimately, *in vivo* studies will be required to demonstrate that the *in vitro–in vivo* correlation assumed is valid and to examine the effect of current application and other formulation variables (such as pH) on the skin of infants and premature neonates.

4. Conclusions

This study demonstrated that cathodal iontophoresis of phenobarbital through intact skin is more efficient than anodal iontophoresis and passive diffusion. Competition from anions present in the donor formulation must be minimized to optimise phenobarbital delivery. The results suggest that both passive and iontophoretic delivery of this drug to premature neonatal and paediatric patients may, in some circumstances, be feasible and attractive. Of course, the costs associated with the development and use of such new transdermal systems must be carefully balanced against their potential to provide an improved and better-tolerated therapy.

Acknowledgement

A. Djabri is grateful to the Algerian Government for sponsoring her Ph.D.

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