Percutaneous lignocaine absorption in newborn infants

D A Barrett, N Rutter

Abstract

The permeability of the skin of newborn infants to lignocaine was examined in vitro using excised skin. Samples were studied from 24 infants of gestational age 25 to 40 weeks and postnatal age 0 to 7 days. Mature skin was relatively impermeable to lignocaine, but the more premature infants showed a marked increase in absorption. There was a strong inverse correlation between gestational age and skin permeability. These findings suggest that topical lignocaine would be an effective local anaesthetic in preterm infants. Calculations indicate that there is negligible risk of toxicity due to systemic absorption.

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Local anaesthetic drugs are poorly absorbed through the skin of adults and children.[1] Penetration sufficient to achieve local anaesthesia is possible by the use of a eutectic mixture of two drugs, lignocaine and prilocaine, but they need to be applied under an occlusive dressing and left for an hour or more.[2] This mixture (Emla cream, Astra) is widely used in children before non-urgent venepuncture and has been shown to provide effective anaesthesia. The prilocaine component, however, causes methaemoglobinaemia.[3] As this is likely to be clinically important in very young children, particularly in preterm infants, Emla is not licensed for use in children less than 1 year of age.

The high permeability of newborn epidermis to many compounds[4] suggests that topical analgesia could be achieved in preterm neonates by the application of a simple aqueous lignocaine solution. A study was therefore designed to examine the percutaneous absorption of lignocaine using an in vitro skin cell technique and excised skin samples. The aim was to calculate whether sufficient absorption of lignocaine occurs to provide anaesthesia without systemic effects.

Patients and methods

SUBJECTS
Skin samples were studied from 24 newborn infants, gestational age 25 to 40 weeks, at a median age of 1 day (range 0 to 7 days). Three adult skin samples were studied for comparison.

MATERIALS
Lignocaine hydrochloride was purchased from Sigma (Poole, Dorset). All other chemicals were of analytical reagent grade or better. Constant ionic strength buffers (0·1 M) were used: sodium acetate/acetic acid (pH 4·0), Sørensen's phosphate buffer (pH 5·6–8), and carbonate/bicarbonate buffer (pH 10·7).

SKIN PREPARATION AND PERMEABILITY EXPERIMENTS
Excised skin from newborn infants was used in the permeation studies. Samples of whole skin of 2–3 cm^2 area were removed at necropsy by abdominal incision within 24 hours of death. The skin samples were wrapped in aluminium foil, sealed in a small plastic envelope, and stored in a freezer at −30°C. Before use the frozen skin sections were thawed at room temperature and rehydrated in phosphate buffered saline (PBS) (pH 5·6 with 0·01% sodium azide to prevent bacterial growth) at 4°C for 18–24 hours 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.

The epidermal layer of adult skin was separated from the whole skin by suspension in water (60°C, two minutes), dried at room temperature (30 minutes), and stored frozen at −30°C before use. The subsequent experimental procedure was identical to that used for excised neonatal skin.

Each skin section was mounted carefully between the half cells of a side by side diffusion cell 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 together and the receptor half cell (inner, dermal side) was filled with buffer (PBS, pH 5·6) and the donor half cell (outer, stratum corneum side) was filled with lignocaine solution (10 mg/ml in 0·1 M acetate buffer pH 4·0 or appropriate buffered pH). The jacketed diffusion cells were maintained at 32°C using a flow heater (Grant Instruments, Cambridge) 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

Department of
Pharmaceutical
Sciences, Nottingham
University,
Nottingham NG7 2RD
B A Barrett

Department of Child
Health, Nottingham
University
N Rutter

Correspondence to:
Dr Barrett.

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Table 1 In vitro permeation of lignocaine through human neonatal skin

Gestational age (weeks)	Postnatal age (days)	Apparent permeability coefficient (cm/hour×10⁻⁴)*	Lag time (hours)	Lignocaine bound to skin (μg/cm²)
25	1	120·0	0·5	–
25	1	62·5	0·7	–
25	1	67·0	<0·5	168
25	s/b	55·0	0·4	72
26	1	388·4	<0·5	425
26	7	8·5	2·6	141
26	7	3·5	0·0	52
30	s/b	19·3	<0·5	40
32	s/b	1·0	0·6	152
32	s/b	27·0	<0·5	486
32	1	6·4	<0·5	112
33	2	6·0	3·7	2632
34	1	6·6	1·9	967
34	1	7·4	1·9	940
34	1	4·4	–	–
35	1	0·7	–	209
35	1	0·3	–	157
36	4	0·5	<0·5	22
36	4	0·9	<0·5	384
36	1	2·9	2·4	101
36	1	2·6	<0·5	26
38	s/b	3·8	<0·5	24
38	s/b	0·5	<0·5	60
40	s/b	0·044	<0·5	133
Adult epidermis samples		0·9		
		3·4		
		0·3		

s/b=Still born. *Single determination for each skin sample.
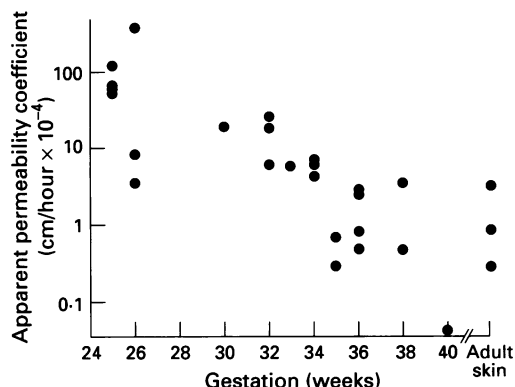
Relation between gestational age and percutaneous lignocaine absorption in newborn infants.

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 hours to remove any lignocaine remaining bound to the skin. The permeation samples and the skin extracts were analysed by high performance liquid chromatography (HPLC) to determine the lignocaine concentration.

ANALYTICAL METHODS
Lignocaine was determined by HPLC under the following conditions: the column was a Hypersil ODS (Shandon Scientific, Runcorn), 15 cm long, 4·6 mm internal diameter with 5 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, the injection volume was 50 μl, and an ultraviolet absorbance detector (wavelength 220 nm) was used. The limit of detection of each compound was 0·02 μg/ml and the assay was linear over the range 0·02 to 100 μg/ml.

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:

$$J_T = AP\Delta C$$

In this equation J_T is the total steady state flux of drug in μg/hour; A is the area of the membrane (0·64 cm²); P is the apparent permeability coefficient in cm/hour 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. The steady state flux was calculated from the slope of the terminal, linear portions of the plot of cumulative lignocaine 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. Lignocaine is present in the donor solution (pH 4·0) predominantly in its ionised form (lignocaine $pK_a = 7·86$). A small but significant amount of the unionised drug will be present, however. The proportion of ionised to unionised drug transported depends on the structure of the skin barrier which, in premature neonates, is known to change with gestational age. Hence no attempt has been made to identify the transported species and the calculated permeability coefficients relate to the total lignocaine flux.

The significance of any correlation between the gestational age of the subjects and the parameters of permeation of lignocaine or lag time was tested using Spearman's rank correlation test for non-parametric data.

Results
Table 1 gives the apparent permeability coefficients (a standardised measure of the permeability of the skin sample) and lag times of lignocaine penetration. A strong inverse relation (p<0·01) exists between the gestational age of the infant and the permeability of skin to lignocaine (figure). The permeability of skin at 25 weeks' gestation was 10² to 10³ times greater than at term. Lignocaine penetration through adult epidermis was similar to that in full term neonates. There was no correlation between gestational age and the amount of lignocaine bound to the skin sample at the end of the in vitro skin cell experiment, and no correlation between gestational age and lag time.

Table 2 shows the effect of pH on the permeation of lignocaine through neonatal skin. There was a threefold increase in the permeability coefficient at alkaline pH, consistent with the predominance of the unionised form of the drug above its pK_a value of 7·86.

Discussion
Lignocaine is known to be poorly absorbed through adult skin and we have showed that it is also poorly absorbed through mature

Table 2 Effect of pH on the permeation of lignocaine through neonatal skin

pH	Apparent permeability coefficient $(cm/hour \times 10^{-4})$	Lag time (hours)	Lignocaine bound to skin $(\mu g/cm^2)$
4·0	2·6	5·1	130
6·8	2·9	5·7	503
8·3	7·9	8·3	1155
10·7	7·2	10·7	1172

All data from a single piece of excised skin from an infant of 36 weeks' gestation, 1 day postnatal age.

newborn skin. It readily penetrates the much weaker epidermal barrier of preterm infants' skin, however. This has been noted with a number of drugs, including theophylline, salicylate, alcohols, and diamorphine.[5-8] The epidermal barrier to drug absorption, the keratinised stratum corneum, is poorly developed in preterm infants in the first weeks of life, during which period the infant may be susceptible to toxicity due to topically applied drugs.[9] The amount of lignocaine which passes through the skin of premature infants is appreciable, and would be sufficient to cause local anaesthesia. It is necessary, however, to consider the potentially toxic effects of the systemically absorbed lignocaine to ensure that the topical lignocaine is safe. It is possible, using a standard pharmacokinetic equation,[10] to estimate the maximum plasma concentration of lignocaine arising from systemic absorption using the permeability coefficients recorded in this study and assuming a mean plasma clearance value of 9·2 ml/min/kg for lignocaine in preterm infants.[11] In the case of an extremely premature infant of 1·0 kg body weight and 25 weeks' gestation, lignocaine solution applied to a skin area of 5 cm² would result in an estimated plasma lignocaine concentration of about 1·0 μg/ml. This is below the therapeutic concentration of lignocaine (1·5–6·0 μg/ml) when used as an antiarrhythmic drug and considerably lower than the toxic concentration. For infants of greater maturity the estimated plasma drug concentration would be proportionately lower. This indicates that topical application of the drug is unlikely to lead to unwanted systemic effects. In more mature infants, with lower skin permeability and greater body weight, the estimated systemic absorption is likely to be negligible.

Two of the skin samples from infants of 26 weeks gestation showed a markedly lower permeation than that observed in the other skin samples from premature infants. It is known that the skin of premature infants matures rapidly to reach maturity within 14 to 21 days.[9] These skin samples were from infants with a postnatal age of 7 days, compared with a median postnatal age of 1 day. It is therefore likely that the skin barrier had partially matured over this postnatal period,

providing an explanation for the lower rate of absorption.

The observed increase in the rate of penetration of lignocaine at alkaline pH is expected as the drug is predominantly in its unionised form above its pK_a value (7·6). It is known that unionised drugs permeate more readily through the lipid barrier of the skin. This result is in agreement with a previous study of the effect of pH on the penetration of lignocaine through adult human stratum corneum, which reported a threefold increase in lignocaine penetration through adult skin at alkaline pH compared with acid pH.[12] This pH dependency of lignocaine penetration could potentially be used as a means of controlling the rate of permeation into skin as the skin has a relatively wide tolerance to pH (between pH 4 and 11).

The data presented on the binding of lignocaine to skin in vitro indicates that the extent of binding is not dependent on gestational or postnatal age. Hence there is no evidence for the formation of a depot of lignocaine in the skin which may cause difficulties with in vivo studies.

In conclusion, this in vitro study suggests that percutaneous absorption in preterm infants is likely to be sufficient to produce local anaesthesia without causing unwanted systemic side effects. The study provides further evidence of the considerably reduced barrier to drug absorption through the skin of preterm newborn infants.

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