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The effect of iontophoresis and vehicle pH on the in-vitro permeation of lignocaine through human stratum corneum

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The effect of iontophoresis and the pH of aqueous vehicles on the rate and extent of permeation of lignocaine through excised human stratum corneum was investigated. In the absence of iontophoresis, the rate of penetration was greatest at the higher pH values where lignocaine exists mainly in the unionized form; iontophoresis was most effective at the lower pH values where lignocaine is mainly ionized. At pH 3·4 and 5·2, the flux increased during iontophoresis, by approximately 8·5 and 4 times, respectively, relative to that occurring without iontophoresis. The present results suggest that some weak electrolytes which show poor percutaneous penetration may be administered topically using iontophoresis provided the drug is kept in a highly ionized form.

Drugs which can be applied to the skin are generally restricted to unionized lipophilic compounds. Ions have been shown to penetrate the skin when an electrical potential gradient is applied as in iontophoresis which is a simple, safe and well documented method of introducing ions or polar substances into the skin by use of a direct current of 40 μA to 1 mA applied through electrodes placed on the surface of the skin (Harris 1967; Wahlberg 1970; Gangarosa et al 1978, 1980; Schaefer et al 1982).

Russo et al (1980) found that when lignocaine was administered topically by iontophoresis to volunteers it produced a local anaesthesia of significantly longer duration than did administration by swabbing, thus avoiding the use of hypodermic needles. The optimal conditions for iontophoretic transport of lignocaine were not determined.

In the present work, the role of pH and the effect of iontophoresis on the rate of in-vitro permeation of lignocaine hydrochloride through human stratum corneum has been investigated.

Materials and methods

Materials. Lignocaine hydrochloride BP was a gift from Astra Pharmaceuticals, Australia. All other reagents were of analytical grade. Constant ionic strength buffers were used; McIlvaine buffer for pH 3·4–8·0 (sodium phosphate/citric acid) and Sörensen's buffer for pH 8·0–12·8 (glycine/sodium hydroxide). Deionized glass-distilled water was used. Tritiated water ($2·5 \times 10^6$ d min^{-1} g^{-1}) was obtained from Packard Instrument Company, USA.

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Permeation studies. Samples of human skin, including the subcutaneous fat, approximately 25 cm by 6 cm, were removed from the mid-abdominal region of a Caucasian male cadaver aged 60 years within 48 h of death and stored at −20 °C. The subcutaneous fat was trimmed and the method of Kligman & Christophers (1963) used to remove epidermis and stratum corneum. The transparent sheet of stratum corneum so obtained was washed three times with water, dried overnight at room temperature (20 °C) and stored at −20 °C. The stratum corneum was allowed to thaw overnight at room temperature and rehydrated by immersion in water for 1 h before being placed in the permeation cell with dermal side towards receptor compartment. It was supported in this position by wire mesh (Fig. 1). A thin film of silicone lubricant (Apiezon-AP100) was spread on the lapped glass surfaces of the cell to provide a water-tight glass to membrane seal.

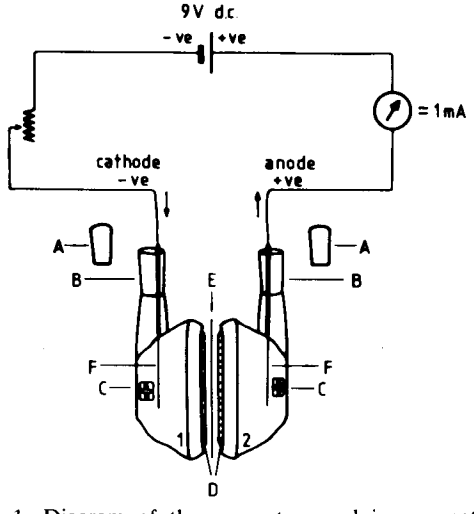

FIG. 1. Diagram of the apparatus used in permeation experiments and iontophoresis. A, glass stoppers; B, sampling ports; C, magnetic fleas; D, wire mesh; E, stratum corneum; F, platinum electrodes. A spring loaded clamp is used to hold the receptor (1) and donor (2) compartments together.

The clamped cells were immersed in a water bath at 25 ± 0·5 °C and a constant stirring rate of 40 rev min^{-1} was maintained in both the compartments using a synchronized motor and external magnets. The maximum capacity of each of the donor and receptor compartments (Fig. 1) was 3·5 ml and the surface area

of stratum corneum exposed to the solution was 4·5 cm^2.

The donor compartment contained a solution (0·1%) of the drug in a buffer at each of six pH values to obtain different degrees of ionization. Isotonic sodium chloride (pH 4·75) was used as the receptor fluid. Samples were taken at regular intervals from the receptor side (0·5 ml) and immediately replaced by the same volume of isotonic sodium chloride. Occasionally, samples (0·1 ml) were also withdrawn from the donor compartment.

The concentrations of lignocaine hydrochloride in aqueous samples were measured spectrophotometrically (λ = 254 nm; Beckman DB-G) by reference to a calibration plot which was linear. The change in the receptor volume during sampling was accounted for when the cumulative amount of lignocaine was calculated. All permeation experiments were run over 6 h and at least in duplicate. Experiments were stopped when the fall in the donor concentration exceeded 10%.

Iontophoresis in-vitro. The cells were placed in the water-bath as above with electrodes located in the donor and receptor compartments during the permeation experiments. As lignocaine is a base, the anode was placed in the donor compartment. Samples from receptor and donor compartment were withdrawn as described above. During permeation experiments, iontophoresis was applied at an elapsed time of 10 min and every hour thereafter for 3 min. The ionic strength of the buffers, the duration of the application of iontophoresis (3 min) and the magnitude of current (~1 mA) which was generated from a standard 9 volts (d.c.) adaptor attached to the mains electric source were kept constant. The 1 mA current used during this work was an arbitrary value which has also been suggested by Schaefer et al (1982). All experiments were performed at least in duplicate. Analysis of lignocaine was carried out as described above.

The integrity of the stratum corneum was examined at the end of each run (with and without iontophoresis) by quantifying the permeation of tritiated water over 3–4 h. The tritiated water was analysed by liquid scintillation counting (Rackbeta II, LK, Wallac, Finland) using Biofluor (New England Nuclear). Visual examination using congo red was also carried out to examine the integrity of the stratum corneum.

Data analysis. The cumulative amount of solute that passes through a membrane from a donor solution has been described using Fick's first law of diffusion (Crank 1975). The steady state flux is the slope of the linear portion of the plot of cumulative amount permeated against time (Fig. 2a). The lag time is defined as the time required for a solute to penetrate the epidermal barrier and corresponds to the time at which the steady state flux is zero. This time is found by extrapolating the steady state portion of the cumulative amount permeated versus time plot to the time axis (Crank 1975).

Results and discussion

Fig. 2a shows the cumulative amount of lignocaine permeated through excised human stratum corneum under various pH conditions and in the presence of iontophoresis. In the absence of iontophoresis the amount of lignocaine passing through the stratum corneum in a given time increases about two fold as the pH changes from 3·4 to 11·7. When iontophoresis was applied, the cumulative amount permeated was increased markedly at lower pH values.

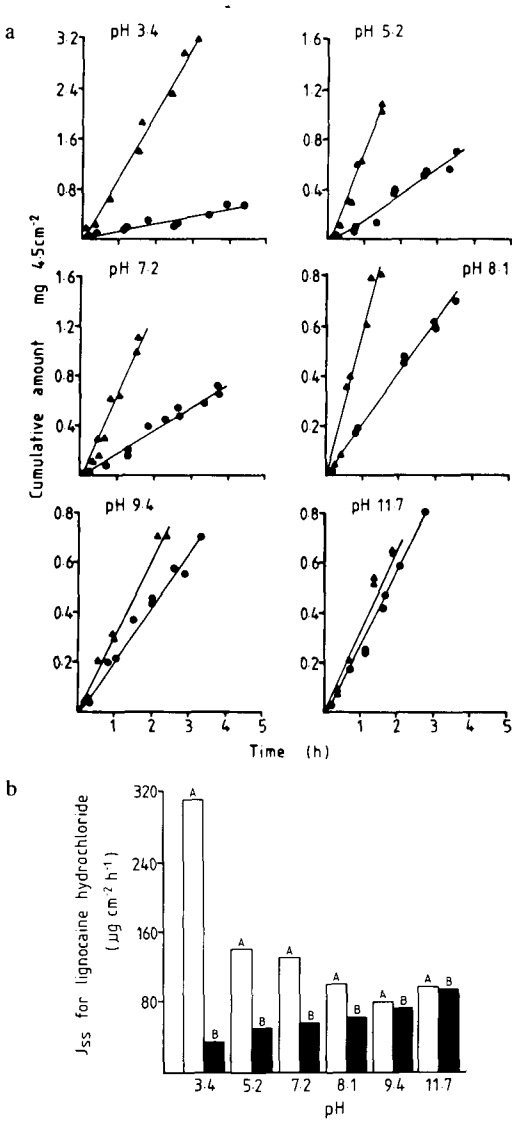

FIG. 2. In-vitro permeation of 0·1% lignocaine hydrochloride through human stratum corneum at 25 °C pK_a (base) = 7·90. a, Cumulative amount versus time. ● Normal, ▲ Iontophoresis. (Curve drawn through data points of two individual runs. The line was drawn by eye.) b, Steady state penetration flux (Jss) versus pH with iontophoresis (A) and without iontophoresis (B).

Fig. 2a also shows that lag time for lignocaine is short (with and without iontophoresis). Blank & Scheuplein (1969) have suggested that the magnitude of lag time may reflect the route of penetration through the skin. Short lag times indicate that the route of penetration may be through the hair follicles and the sweat ducts (shunts) while long lag times may reflect the transport of solute through the cells (intracellular) of the stratum corneum. The shorter lag times of lignocaine (with and without iontophoresis) indicates the possible transport of ionized species through shunts. However, this transport of ionized species is likely to be enhanced with further reduction in lag times during the application of iontophoresis (Fig. 2a).

Fig. 2b shows that at pH 3·4 and 5·2, the flux is increased during iontophoresis by approximately 8·5 and 3·5 times, respectively, whereas at pH 11·7, the increase in the rate of permeation of lignocaine due to iontophoresis is negligible. The flux of lignocaine, without iontophoresis, increases with pH (decrease in ionization). The different fluxes (Fig. 2b) reflect the dependence of the rate of penetration on ionization.

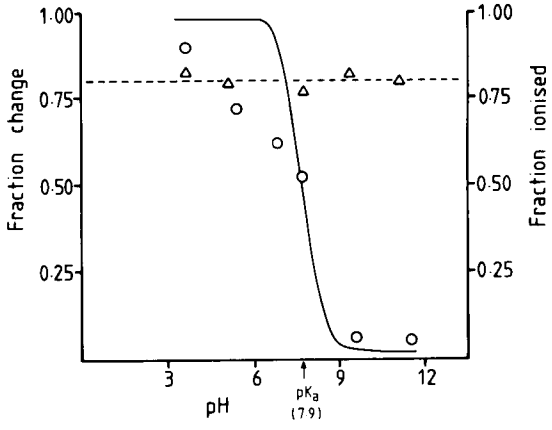

FIG. 3. Effect of vehicle pH on the change in the steady state flux for lignocaine hydrochloride (○) and tritiated water (△) through human stratum corneum. The fraction of lignocaine hydrochloride ionized is indicated by the solid line. The fraction change in flux was defined as the ratio; (flux during iontophoresis—normal flux)/flux during iontophoresis.

Fig. 3 shows that the fraction change in flux for lignocaine hydrochloride during iontophoresis is related to the degree of ionization. As the extent of ionization increases, the flux also increases. A standard one-way analysis of variance was performed with a factorial arrangement of treatments, where the factors are pH and fluxes (with and without iontophoresis) using a statpak (Teddybear; a statistical program written by J. B. Wilson, Botany department, University of Otago, Dunedin, New Zealand). The log transformation of fluxes was required to equalize variances between treatments (Snedecor & Cochran 1980). The fluxes for six pH values revealed a significant difference ($F = 295{\cdot}3$, $df = 5/12$, $P < 0{\cdot}001$) between the fluxes with and without iontophoresis and was dependent upon the degree of ionization. A test of least significant difference (Snedecor & Cochran 1980) also showed that between pH values of 3·4 and 8·1 there was evidence of significant difference in fluxes with and without iontophoresis (lsd, $P < 0{\cdot}05$) whereas at pH values of 9·4 and 11·7 the change in magnitude of flux due to iontophoresis is not significant (lsd, $P > 0{\cdot}05$). This statistical analysis shows that when lignocaine is in a highly unionized form (pH 9·4 and 11·7) the effect of iontophoresis on the rate of penetration of the substance is negligible.

The exposure of the stratum corneum to aqueous solutions of different pH values with and without iontophoresis did not have any effect on the permeability of tritiated water. The fraction change in flux for water was independent of pH for the stratum corneum specimens which were exposed to solutions of different pH values (Fig. 3).

Iontophoresis is the transfer of ions through the stratum corneum in the presence of an electric current (Harris 1967). The relation between the rate of permeation of lignocaine during iontophoresis and the degree of ionization (Fig. 3) may possibly be related to the conductivity of lignocaine in aqueous solutions (Gangarosa et al 1978). Gangarosa et al (1978) also reported that the conductivity of lignocaine decreased at high pH values (decrease in ionization of the base) and that the hydrochloride salts of local anaesthetics conducted best at pH 5·0 where all local anaesthetic molecules exist in the positively charged form.

Clinically, a pH of 5 to 6 (pH of the epidermis 4·2–6·5; from Katz & Poulsen 1971) would be suitable for lignocaine solutions to be applied to the skin with iontophoresis as this pH range would give sufficient conductivity for iontophoresis to be effective and safe. It has been reported by Russo et al (1980) that lignocaine was effective at pH 6·0 when administered iontophoretically to volunteers.

The present work shows that the increased anaesthetic effect found for lignocaine during iontophoresis (Russo et al 1980) was pH dependent. It may be possible that other weak electrolytes which show poor percutaneous absorption could also be administered by iontophoresis provided the drug was applied in a highly ionized form.

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Effect of phenobarbitone on the distribution and elimination of imipramine in rats

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The effect of phenobarbitone on the steady state volume of distribution (Vd_{ss}) and the total body blood clearance ($CL_{tot,b}$) of imipramine and the serum concentration of its metabolite, desipramine was examined. The serum disappearance of imipramine after an 8 mg kg^{-1} i.v. dose followed a biexponential decline in both control and phenobarbitone-treated rats while the concentration of its metabolite increased in the phenobarbitone-treated rats then rapidly declined compared with that in control rats. Since $CL_{tot,b}$ was nearly equal to the hepatic blood flow (Q_H), Q_H may be the rate-determining step of imipramine elimination. In the control rats the Vd_{ss} of imipramine was large at 19·9 litre kg^{-1}. In the phenobarbitone-treated rats the pharmacokinetic parameters, biological half-life ($t_{\frac{1}{2}}$) and Vd_{ss} significantly decreased to approximately 23–40% while $CL_{tot,b}$ increased to 126% of those in the control rats, although the latter difference was not statistically significant. The blood-to-plasma concentration ratios (R_B) of imipramine and desipramine decreased in the phenobarbitone-treated rats. The urinary excretion ratios of imipramine and desipramine, to the dose of imipramine over 8 h, were <1·5% in both groups. These ratios were not significantly changed in the phenobarbitone-treated rats. It was concluded that the significant decrease in $t_{\frac{1}{2}}$ of the phenobarbitone-treated rats may not be attributed to the changes in $CL_{tot,b}$ and/or in the urinary excretion, but mainly to the decrease in Vd_{ss}.

The concomitant administration of barbiturates to patients receiving tricyclic antidepressants has been observed to result in a decreased plasma level of antidepressant (Hammer et al 1967; Burrows & Davies 1971; Silverman & Braithwaite 1972; Ballinger et al 1974). Similarly, in normal subjects, a decrease in steady state plasma concentration of nortriptyline was observed on coadministration of additional drugs including barbiturates (Alexanderson et al 1969). Though these phenomena are likely to be due to enzyme induction which produces the enhanced metabolic elimination of antidepressants, the literature is lacking in animal experiments to support this. Also reports concerning the effects of barbiturates on the tissue distribution of weakly basic drugs, including tricyclic antidepressants (Breyer-Pfaff et al 1978), are few. We have examined the effect of continuous phenobarbitone administration on the time-courses of imipramine and its metabolite, desipramine, and on their pharmacokinetic parameters, i.e. biological half-life ($t_{\frac{1}{2}}$), total body blood clearance ($CL_{tot,b}$), volume of distribution (Vd) and urinary excretion ratio, in rats.

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Methods

Imipramine HCl, desipramine HCl and sodium phenobarbitone were kindly supplied by Yoshitomi Pharm. Co., Tokyo, Japan, Ciba Geigy Pharm. Co., and Sankyo Pharm. Co., respectively. [^{14}C]Imipramine (48 mCi mmol^{-1}) was purchased from the Radiochemical Research Centre (Amersham, UK); [^{3}H]-imipramine and [^{3}H]desipramine were purchased from New England Nuclear Co., Boston, Mass. These radioactive compounds were at least 98% pure by TLC. All other reagents were commercial products and of analytical grade.

Adult, male Wistar rats, 200–290 g, were used. They had free access to tap water and standard laboratory chow (CE-2, Clea Japan Inc., Tokyo, Japan) except before i.v. administration of imipramine when they were fasted for 24 h. For the phenobarbitone treatment, the animals received 1 g litre^{-1} in their drinking water for 6 days. Control rats received water. Under light ether anaesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing. The urinary bladder was cannulated with PE260 polyethylene tubing. Cannulated rats were kept in restraining cages with water, under normal housing conditions before experiments. After 3 h for recovery from ether anaesthesia, the rats were given 8 mg kg^{-1} of imipramine containing 40 μCi kg^{-1} of [^{14}C]imipramine in saline through the femoral vein cannula over 30 s. Blood samples (0·25 ml) then were obtained at 1, 2, 5, 10, 15, 30, 60, 120, 180, 240, 360 and 480 min in polyethylene centrifuge tubes. Urine was collected for 8 h. Body temperature was kept at 37 °C using a heat lamp. Serum was separated by