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# Percutaneous absorption of azidoprofen, a model for a soft anti-inflammatory drug for topical application

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## Summary

The penetration of azidoprofen through excised hairless mouse skin was investigated. Formulation factors influencing skin permeation, such as pH, solute and cosolvent concentration were studied and were related to physico-chemical parameters including $pK_a$, solubility and partition. In addition, the effect of a range of penetration enhancers on the transport of azidoprofen was also assessed. The flux of azidoprofen from solution formulations was dependent on vehicle pH and permeant concentration and was directly related to the degree of ionisation of the solute. Increased permeant concentration in the vehicle resulted in a greater level of saturation and, hence, a higher rate of penetration. In contrast, over the range used (0–30%), propylene glycol concentration in the vehicle had little effect. Flux from suspensions was independent of pH, since the level of unionised drug, the predominant diffusing species, was maintained at the intrinsic saturated solubility at all pH values. Pretreatment of the skin with a range of enhancers only moderately promoted permeation. Pretreatment with Azone in propylene glycol resulted in an increased flux with increasing pH and thus appeared to facilitate penetration of the ionised species.

## Introduction

Inflammation is a major component of many skin diseases and non-steroidal antiinflammatory drugs (NSAIDs) have potential in dermatology (Greaves, 1987). The altered arachidonic acid (AA) metabolism which is characteristic of psoriatic skin has led to attempts to inhibit the inflammatory responses accompanying the disease. Cyclooxygenase inhibition, the mechanism of action of many NSAIDs, limits enzymatic transformation of AA to prostaglandin metabolites. However, cyclooxygenase inhibitors have not found a major role in the treatment of inflammatory dermatoses with the exception of UVB-induced erythema, where the early elevation of $PGE_2$ levels may be inhibited by indomethacin (Kobza Black et al., 1978). Indeed, cyclooxygenase inhibitors aggravate a number of dermatoses (Griffiths et al., 1985). Leukocyte accumulation is not suppressed by these drugs due to the continued production of chemotactic lipoxygenase products, and suppression of the alternative lipoxygenase

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pathway is also of importance. Dual inhibitors may be of significant therapeutic value, since depression of one pathway is often compensated by substrate diversion to another.

Significant improvement in psoriatic skin lesions during benoxaprofen therapy (Allen and Littlewood, 1982, 1983; Kragballe and Herlin, 1983) demonstrated its effectiveness in psoriasis and in palmoplantar pustulosis (Fenton and Wilkinson, 1982). Cystic acne and nodular prurigo, which are typified by neutrophil infiltration and epidermal hyperplasia, have also been shown to respond to benoxaprofen therapy (Hindson et al., 1982a, b). It has been proposed that benoxaprofen differs in its mode of action from other NSAIDs due to its weak cyclooxygenase inhibitory activity (Cashin et al., 1977) and the inhibition of arachidonate lipoxygenase, a possible cause of its anti-psoriatic activity, in isolated leukocytes has been demonstrated (Walker and Dawson, 1979; Harvey et al., 1983) but questioned (Masters and McMillan, 1984; Salmon et al., 1984). The inhibition of monocyte migration (Meacock et al., 1979) may also contribute to its antipsoriatic action, since these features are enhanced in patients with psoriasis (Czarnetzki, 1983) and a normalisation of in vitro monocyte chemotaxis in patients receiving benoxaprofen has been demonstrated prior to clinical resolution of the lesions (Kragballe et al., 1985). Other antipsoriatic therapies have also been shown to possess lipoxygenase inhibitory activity including dithranol (Bedford et al, 1982) and retinoids (Bray, 1984). A high incidence of photosensitivity, onycholysis and hepatotoxicity as well as a number of fatalities, particularly in elderly patients suffering from renal or hepatic failure, resulted in the withdrawal of benoxaprofen from clinical use. The therapeutic mechanism of this potential anti-psoriatic drug therefore remains unresolved but efforts to develop lipoxygenase inhibitors continue (Chan et al., 1987; Degreef et al., 1990).

A strategy to overcome such toxicity problems is the development of soft drugs (Bodor, 1982), compounds which are active in themselves but which undergo predictable metabolic inactivation to non-toxic products. Additionally, topically applied drugs penetrate local subcutaneous structures (Marty et al., 1985) and a number of studies show preferential accumulation of NSAIDs in deeper tissues following dermal delivery (Rabinowitz et al., 1982; Reiss et al., 1986). To combine these approaches, we have previously described azidoprofen (2-(4-azidophenyl)propionic acid, AZP) as a model for a topical soft drug undergoing deactivation by reduction to an inactive amino analogue (Naik et al., 1993a,b). Here, we report the results of an in vitro assessment of its topical delivery profile.

## Experimental

*Apparatus*

HPLC analyses were performed using a system constructed from an Altex 100A dual reciprocating, solvent-metering pump which delivered mobile phases at a flow rate of 1 ml $min^{-1}$ to a stainless-steel column (10 cm × 4.6 mm) packed with 5 $\mu$m Hypersil-ODS (Shandon, U.K.) reversed-phase material. Samples were introduced through a Rheodyne 7120 injection valve fitted with a 10 $\mu$l loop and UV detection was accomplished at 250 nm and 0.08–1.28 AUFS with a Pye Unicam LC3 variable-wavelength UV detector equipped with an 8 $\mu$l flow cell. Chromatograms were recorded using a JJ instruments CR452 chart recorder operated at a chart speed of 12.0 cm $h^{-1}$. The mobile phase comprised aqueous acetonitrile (50%) containing 0.1% diethylamine with the pH adjusted to 2.5 with orthophosphoric acid and the retention time of AZP under these conditions was 3.1 min. For caffeine ($t_R$ = 5 min) the mobile phase comprised 10% acetonitrile and theophylline ($t_R$ = 3 min) was used as internal standard (7.5 $\mu$g $ml^{-1}$ final concentration).

*Transport studies*

Skin was obtained from male hairless mice (type MF1, supplier Olac Ltd), 2–6 months old and weighing 20–30 g. To minimise problems with this tissue (Bond and Barry, 1988a, b; Hinz et al., 1989) studies were conducted for usually 12–24 h. The mice were killed by cervical dislocation and whole thickness intact skin carefully

excised and placed in ice-cold phosphate-buffered saline (PBS). After removal of any subcutaneous fat or visceral debris from the undersurface, the membranes were washed with PBS and either used immediately or stored in the frozen state, between sheets of aluminium foil for up to 12 h. Each epidermal specimen generally provided sufficient skin for three or four diffusion cells, each portion measuring approx. 2.5–3.0 cm in diameter. These samples were distributed amongst the experiments, which were designed such that each set of replicate permeability values was obtained from at least two and usually three donors, thus minimising inter-specimen variation. A series of experiments also utilised a silicone rubber membrane (Silastic Medical grade 500-3, Dow Corning, 0.010 inch thick). Membranes were mounted (epidermis uppermost for skin) between the two halves of a jacketed Franz-type diffusion cell (Franz, 1975), with a surface area of approx. 2.0 $cm^2$ for diffusion. The two halves of the cell were secured with Nescofilm and held together by a spring clamp. The receptor compartment, which had a capacity to hold 20–30 ml of fluid, could be sampled via a side arm. Both donor compartment and side-arm were covered to minimise evaporation. The receiver phase was stirred and the cell was maintained at 37°C resulting in a skin surface temperature of $32 \pm 1$°C.

*Test vehicles*

A range of non-buffered (10–50% v/v PG in water) and buffered aqueous propylene glycol (PG) mixtures (10% v/v pH in water; pH 3.50–7.40) were used as test vehicles. The latter were prepared from concentrated citric acid-phosphate (McIlvaine) buffers of constant ionic strength (pH 3.0–7.0) (Perrin and Dempsey, 1974) which were diluted 10-fold with the incorporation of PG (10% v/v). pH values were adjusted as required with sodium hydroxide or phosphoric acid and values quoted are those of the final mixture. Donor phases consisted of either solutions, saturated solutions or suspensions of azidoprofen in the appropriate vehicle. Suspensions were prepared by stirring an excess (3–4 times saturated solubility) of AZP in vehicle (10 ml) in a sealed container, protected from light, for 24 h at 32°C. Saturated solutions were obtained by filtration through a 0.2 $\mu$m membrane (Millipore GS). Suspensions of caffeine in 10% PG buffer were prepared similarly. Penetration enhancer pretreatment solutions were prepared by dissolving the enhancer to the required concentration (% v/v or % w/v) in the appropriate solvent. Enhancers used were 3% v/v Azone in PG, 3% v/v Azone with 0.1% Tween 20 in normal saline (TS), 5% v/v oleic acid in PG, 15% w/v decyl methyl sulphoxide (DCMS) in PG, 0.5% w/v dodecylamine (DCA) in PG. Control experiments using propylene glycol and 0.1% Tween 20 in normal saline as pretreatment solutions were also conducted.

*Permeation procedure*

With the appropriate membrane mounted, receptor chambers were filled with the chosen solvent (15–30 ml, accurately measured), which corresponded to drug-free donor vehicle, maintained at 37°C and de-gassed by sonication to minimise volume changes and prevent accumulation of air bubbles at the skin-receptor fluid interface. The membrane was allowed to equilibrate with the receptor solution for 1 h and air bubbles in the vicinity of the membrane were removed via the sampling port by carefully rocking the cell assembly. An aliquot (5 ml for solutions; 3 ml for suspensions) of the formulation under study was introduced into the donor chamber. Samples of the receptor phase (1 ml) were removed at appropriate intervals (usually hourly) and were assayed by HPLC following suitable dilution. After the withdrawal of each sample the receptor fluid was replenished with an aliquot of the drug-free vehicle. To correct for progressive dilution of the receptor phase the mass of drug transported across the membrane ($M_t$) at time $t$ was calculated from:

$$M_t(n) = V_r \cdot C_n + V_s \cdot \sum_{m=1}^{n-1} C_m \qquad (1)$$

where $M_t(n)$ is the current, cumulative mass transported across the membrane at time $t$, $C_n$ represents the current concentration in the re-

ceptor medium and $\Sigma C_m$ denotes the summed total of the previous measured concentrations ($m = 1$ to $(n-1)$). $V_r$ is the volume of the receptor medium and $V_s$ corresponds to the volume of sample removed for analysis.

For studies involving pretreatment procedures, the pretreatment solution (1 ml) was applied to the epidermal surface of the skin for 1 or 12 h prior to introduction of the test formulation. At the end of this period, the application was removed by gently blotting with a paper tissue. The skin surface was then briefly rinsed with distilled water and blotted dry prior to addition of the donor medium. All permeation studies were performed in triplicate and were protected from light for the duration of the study.

*Solubility determinations*

The solubility of AZP in each vehicle employed was determined in triplicate by suspending an excess of AZP (25–50 mg) in the appropriate solvent system (5 ml) in a stoppered glass sample tube at 32°C. These suspensions were stirred for 48 h, stood for 2–3 h and an aliquot of the supernatant fluid was withdrawn and filtered through a 0.2 $\mu$m membrane (Millipore GS). The filtrate was diluted with PG to yield a cosolvent concentration of 50% v/v and all further dilutions (to give analytical concentrations of 0.1–1.0 mM) were performed with PG (50%). Concentrations of AZP were determined by assaying 10 $\mu$l aliquots of the resulting filtrate by HPLC.

*Partition coefficient measurements*

*Isopropyl myristate-water* Oil-water partition coefficients for AZP between equal volumes of mutually saturated isopropyl myristate (IPM) and aqueous buffer (citric acid-phosphate (Perrin and Dempsey, 1974) of constant ionic strength (0.5 M)) were determined at 37°C over a pH range of 3.20–7.40. Stoppered sample tubes containing equal volumes (5 ml) of aqueous buffer and a solution of AZP in IPM (100 $\mu$g ml$^{-1}$) were shaken mechanically in a water bath at 37°C for 12 h. After centrifugation (500 $\times g$, 10 min) to ensure separation of the two phases, an aliquot of the aqueous phase was diluted (1:1–1:19) with IPM-saturated water and treated with internal standard solution (1:1; butyl paraben; 10 $\mu$g ml$^{-1}$) and assayed for AZP content by HPLC (10 $\mu$l). The experiments were performed in triplicate.

*Octanol-water* Octanol and aqueous PG (20% v/v) solutions, with or without 0.1 M HCl, were presaturated with each other in the ratio 40:1 at room temperature. This volume ratio corresponds to those used in the partitioning experiments and maintains the distribution of PG between the two immiscible solvents. Stock solutions of AZP (4 mg ml$^{-1}$) were prepared in appropriately presaturated octanol. Stoppered flasks containing 200 ml of aqueous PG (20%), octanol (4.5 ml) and ester stock solution (0.5 ml) were manually inverted at frequent intervals for a period of 1 h, at room temperature, to establish equilibrium. After separation of the two phases by centrifugation, both layers (100 $\mu$l) were analysed by HPLC following dilution (aqueous, 1:1 with methanol; octanol, 500–1000:1 with methanol and octanol-saturated aqueous PG (20%), 3:1) and internal standard (1:1; 1 $\mu$g ml$^{-1}$ in the same solvent). All experiments were performed in triplicate.

The apparent octanol-water partition coefficient of AZP over a range of pH values was determined by mutual saturation of citric acid-phosphate buffers in the pH range 4.20–7.40 (Perrin and Dempsey, 1974) and octanol at 37°C. Stoppered sample tubes containing 5 ml of aqueous buffer and 5 ml of octanol containing AZP (400 $\mu$g ml$^{-1}$) were magnetically stirred in a jacketed beaker maintained at 37°C for 12 h. The two phases were separated by centrifugation (500 $\times g$, 10 min) and diluted appropriately with methanol to yield a final MeOH concentration of 75% v/v. Both phases were then diluted (aqueous, 1–99:1; octanol, 1:149) with internal standard solution (butyl paraben, 2.5 $\mu$g ml$^{-1}$) prior to HPLC analysis (100 $\mu$l). A similar experiment was conducted at 20 $\pm$ 1°C. All determinations were performed in triplicate.

*Skin-vehicle* The epidermis was separated from the hairless mouse skin samples by immersing the membrane in water at 55°C for 30 s followed by ice-cold PBS. The dermis was then removed with a sharp scalpel blade. Quantities of epidermis were then pretreated with the vehicles

employed in the permeation studies by immersing in the vehicle for an appropriate period of time (1 or 12 h) followed by rinsing in distilled water and PBS. Segments of skin (pretreated or untreated as required) were then blotted dry on filter paper and accurately weighed (150–200 mg) into glass sample tubes. Aliquots (1 ml) of AZP solution (200 $\mu$M) in the appropriate vehicle were introduced into the sample tube and the contents gently mixed in a water bath at 37°C for 60 h. Samples of the aqueous layer were diluted (1–4:1) with solvent and internal standard solution (butyl paraben, 20 $\mu$g ml$^{-1}$ in 10% PG, 1:1) prior to HPLC analysis (20 $\mu$l). The amount of AZP taken up by the skin was estimated by calculating the difference between the initial and final concentration in the aqueous layer. Densities of the AZP solutions were determined using 25 ml density bottles to enable partition coefficients to be calculated on a weight basis. All determinations were performed in triplicate and controls without skin were included to eliminate loss due to uptake by glass. Partition coefficients were defined as $P$ = (mass of solute per g of skin)/(mass of solute per g of vehicle). Ionisation constants for AZP, both in aqueous media and 10% aqueous propylene glycol were obtained by potentiometric titration of AZP (10 ml, 0.0025 M) with NaOH (0.05 M) according to Albert and Serjeant (1984).

## Results and Discussion

The saturated solubilities of AZP in aqueous propylene glycol and as a function of pH are summarised in Tables 1 and 2 and show the dependence expected of a weak acid (p$K_a$, 4.29 (water), 4.36 (10% PG)). Partition data are recorded in Table 3 where partition coefficients for the unionised ($P_u$) and ionised ($P_i$) species have been derived from the apparent partition coefficient ($P_{app}$) dependent upon pH (Irwin and Li Wan Po, 1979; Irwin et al., 1990a, 1990b). Over the range of interest, there was no significant effect on the partitioning into skin due to increasing the propylene glycol concentration in the aqueous phase and a mean apparent partition coefficient of 1.1 ± 0.08 ($n$ = 12) was found. Table 4 records data for the partitioning of AZP between 10% propylene glycol buffer (pH 4.50 or 6.50) and hairless mouse skin pretreated with an enhancer or control regimen (for 1 or 12 h as stated). The pretreatment procedure was analogous to that performed in subsequent permeation experiments.

TABLE 1

*Effect of propylene glycol (PG) concentration on the saturated solubility of AZP in buffered vehicles (pH 4.50), at 32°C*

| pH | AZP solubility ($\mu$mol ml$^{-1}$) |
|---|---|
| 3.50 | 11.50 (0.93) |
| 4.00 | 16.44 (0.42) |
| 4.50 | 20.73 (0.38) |
| 5.00 | 29.55 (0.68) |
| 5.50 | 39.20 (0.98) |
| 6.50 | 54.64 [a] |
| 7.40 | 123.1 [a] |

Figures in parentheses are standard errors on the mean; [a] $n$ = 2.

Azidoprofen was found to permeate hairless mouse skin readily from an aqueous PG (10%) vehicle buffered to a pH of 4.50. The solubility of AZP under these conditions was 20.73 $\mu$mol ml$^{-1}$ and, as indicated in Table 5, as the concentration increases towards saturation and hence maximal activity, the rate of penetration increases. In contrast, the values of permeability coefficients, which depend upon diffusion, partition coefficients and membrane thickness ($K_p = D \cdot P/h$), remain essentially constant with a mean (SE) value of 0.0406 ($1.538 \times 10^3$) cm h$^{-1}$. Analogous permeation data from a series of buffered suspensions (pH 4.50) with increasing PG concentrations are shown in Table 6. Flux values remain fairly constant as the fraction of propylene glycol in the vehicle increases whereas permeability coefficients decrease. The solubility of AZP in the vehicle doubles over the range examined here (Table 1) and this is not reflected in an increase in flux. Moreover, the partition coefficient changes little over this range. This implies that PG inhibits transport by influencing either membrane thickness or diffusion coefficient.

TABLE 2

*Effect of pH on the saturated solubility of AZP in 10% propylene glycol-buffer at 32°C*

| Propylene glycol (%) | AZP solubility ($\mu$mol ml$^{-1}$) |
|---|---|
| 0 | 17.25 (0.50) |
| 10 | 20.73 (0.38) |
| 20 | 28.09 (0.23) |
| 30 | 39.24 (3.61) |
| 40 | 76.79 (4.62) |
| 50 | 159.3 (3.18) |

Figures in parentheses are standard errors on the mean.

TABLE 3

*Partitioning data for AZP between various systems*

| Organic phase | Aqueous phase | Temperature (°C) | $P_u$ | $P_i$ |
|---|---|---|---|---|
| Octanol | buffer | 20 | 269.3 | 0.98 |
| | | 37 | 380.2 | 4.18 |
| IPM | buffer | 37 | 57.4 | [a] |
| Epidermis | PG in buffer (10%) | 37 | 6.84 | 1.11 |
| Epidermis pretreated with Azone (3%, 12h) | PG in buffer (10%) | 37 | 37.4 | 1.33 |

[a] No partition of ion into the organic phase.

To evaluate the effect of pH on the permeation of AZP through hairless mouse skin, transport from a series of suspensions was investigated and data are summarised in Table 7. These show that the penetration rates of AZP from suspensions do not vary significantly as pH increases from 3.50 to 7.40 and provide a mean flux of 2.1818 (0.0781) $\mu$mol cm$^{-2}$ h$^{-1}$. Under these conditions, the amount of unionised drug in solution is effectively constant at any pH, and hence the degree of saturation and thermodynamic activity is maintained at unity. Flux values are, therefore, largely independent of the degree of ionisation as permeation through skin is predominantly dependent upon the neutral molecule. In contrast, permeability coefficients decrease with increasing pH (Table 7). Under these conditions these values are effectively apparent permeability coefficients ($K_{app}$) and might be expected to depend upon the apparent partition coefficient ($P_{app}$) according to Eqn 2:

$$K_{app} = \frac{P_{app} \cdot D_{app}}{h} = \frac{(1-\alpha) \cdot P_u \cdot D_u + \alpha \cdot P_i \cdot D_i}{h} \quad (2)$$

where subscripts u and i refer to unionised and ionised molecules, respectively, and the degree of ionisation ($\alpha$) is given by $[H_3O^+]/([H_3O^+] + K_a)$.

TABLE 4

*Partitioning data for AZP between hairless mouse skin, pretreated for 12 h with various regimens, and 10% PG-buffer at pH 4.50*

| Pretreatment | Partition coefficient |
|---|---|
| Tween 20 (0.1%) in normal saline | 4.858 (0.354) |
| Azone (3%) in Tween 20 (0.1%) in normal saline | 7.293 (0.411) |
| Propylene glycol [a] | 4.138 (0.251) |
| Propylene glycol [b] | 1.550 (0.157) |
| Propylene glycol | 4.295 (0.181) |
| Azone (3%) in propylene glycol | 16.54 (1.49) |
| Dodecylamine (0.5%) in propylene glycol [a] | 3.783 (0.241) |
| Dodecylamine (0.5%) in propylene glycol [b] | 2.475 (0.118) |
| Dodecylamine (0.5%) in propylene glycol | 5.970 (0.537) |
| Oleic acid (5%) in propylene glycol | 7.122 (0.199) |

[a] 1 h pretreatment.

[b] pH, 6.50.

Figures in parentheses are standard errors.

TABLE 5

*Permeation data for AZP across hairless mouse skin from solutions of varying concentration in 10% PG buffer at pH 4.50*

| AZP concentration ($\mu$mol cm$^{-3}$) | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | $K_p$ (cm h$^{-1}$) | Lag time (h) |
|---|---|---|---|
| 6.0 | 0.236 (0.015) | 0.0394 (0.0026) | 3.29 (0.65) |
| 9.0 | 0.330 (0.036) | 0.0366 (0.0040) | 1.70 (0.41) |
| 12.0 | 0.518 (0.034) | 0.0432 (0.0028) | 0.81 (0.24) |
| 15.0 | 0.649 (0.037) | 0.0433 (0.0025) | 2.78 (0.07) |

Values in parentheses are the standard errors on the mean.

Assuming the ionic contribution is negligible ($D_u \cdot P_u \gg D_i \cdot P_i$) this approximates to Eqn 3:

$$K_{app} = \frac{(1-\alpha) \cdot P_u \cdot D_u}{h} \quad (3)$$

Thus, a plot of $K_{app}$ against $(1-\alpha)$ should be linear and linear regression analysis gives $K_{app} = 0.033 + 0.146 P_u D_u/\text{h}$ ($r^2 = 0.898$).

These relationships are reinforced by the dependence of skin permeability on drug partitioning where $K_p$ is linearly dependent upon $P_{app}$ as described by the equation:

$$K_p = 0.0174 + 0.0243 P_{app} \quad (r^2 = 0.923) \quad (4)$$

In contrast, the effect of pH on the percutaneous absorption of AZP from equimolar solutions in buffered 10% PG buffer vehicles was significant. Steady-state fluxes, permeability coefficients and lag times are recorded in Table 8 which illustrates the pH dependency of these parameters. Both the flux and the permeability coefficients decrease with increasing pH while lag times increase. The increase in lag time with increasing donor vehicle pH may be due to a progressive change in the apparent diffusion coefficient ($D_{app}$) from $D_u$ to $D_i$ as ionisation progresses. Data using silastic as the membrane show a similar trend and indicate that the pH dependency is not a result of a direct insult to the barrier properties of the skin. Partitioning into skin is favoured by the undissociated form of the drug (Table 3), the proportion of which increases with decreasing pH for an acidic drug such as AZP. The flux of AZP across both membranes is, therefore, related to the fraction of drug in the unionised form $(1-\alpha)$ given by $K_a/([H_3O^+] + K_a)$. For silastic this is (Eqn 5):

$$J = 4.0854 \times 10^{-2} + 0.39303(1-\alpha) \quad (r^2 = 0.998) \quad (5)$$

TABLE 6

*Effect of increasing propylene glycol concentration on the permeation of AZP across hairless mouse skin from suspensions in aqueous PG (pH 4.50)*

| Propylene glycol (% v/v) | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | $K_p$ (cm h$^{-1}$) | Lag time (h) |
|---|---|---|---|
| 0 | 2.111 (0.073) | 0.122 (0.004) | 1.30 (0.04) |
| 10 | 1.974 (0.050) | 0.0952 (0.002) | 2.14 (0.08) |
| 20 | 1.885 (0.137) | 0.0671 (0.005) | 2.52 (0.10) |
| 30 | 2.115 (0.167) | 0.0539 (0.004) | 2.51 (0.167) |

Values in parentheses are the standard errors on the mean.

TABLE 7

*Permeation data for AZP across hairless mouse skin from a series of suspensions in 10% PG buffer*

| pH | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | $K_p$ (cm h$^{-1}$) | Lag time (h) |
|---|---|---|---|
| 3.50 | 1.894 (0.510) | 0.165 (0.004) | 1.63 (0.23) |
| 4.00 | 2.032 (0.199) | 0.124 (0.001) | 1.70 (0.16) |
| 4.50 | 1.974 (0.050) | 0.0952 (0.002) | 2.14 (0.08) |
| 5.00 | 2.031 (0.067) | 0.0687 (0.0023) | 2.08 (0.18) |
| 5.50 | 2.658 (0.167) | 0.0678 (0.0043) | 1.90 (0.15) |
| 6.50 | 1.980 (0.093) | 0.0362 (0.0017) | 0.82 (0.16) |
| 7.40 | 2.609 (0.075) | 0.0021 (0.0061) | 1.62 (0.10) |

Values in parentheses are the standard errors on the mean.

TABLE 8

*Permeation data for AZP across hairless mouse skin from a series of equimolar (15 mM) solutions in 10% PG buffer*

| pH | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | $K_p$ (cm h$^{-1}$) | Lag time (h) |
|---|---|---|---|
| 4.00 | 1.034 (0.026) | 0.0689 (0.0017) | 1.83 (0.16) |
| 4.50 | 0.649 (0.037) | 0.0433 (0.0025) | 2.78 (0.07) |
| 5.00 | 0.362 (0.003) | 0.0241 (0.0002) | 3.39 (0.21) |

Values in parentheses are the standard errors on the mean.

and for skin (Eqn 6):

$$J = 0.110 + 1.321(1 - \alpha) \quad (6)$$

The relative rates of percutaneous absorption (fluxes) of the unionised and ionised forms of a permeant may be estimated from the dependence of flux on pH (Irwin et al., 1990a) using Eqn 7:

$$\frac{J_{app}}{\alpha} = J_u \cdot \frac{(1-\alpha)}{\alpha} + J_i \quad (7)$$

A plot of $J_{app}/\alpha$ against $(1-\alpha)/\alpha$ provides a linear relationship with the equation:

$$J_{app}/\alpha = 1.421(1-\alpha)/\alpha - 9.9262 \times 10^{-2}$$

$$(r^2 = 1.000) \quad (8)$$

The small negative intercept suggests that the ionised form penetrates the membrane but poorly. This is in agreement with the suspension data which correlated well with the unionised component only.

The effects of a number of established penetration enhancers (Goodman and Barry, 1986; Barry and Bennett, 1987; Bond and Barry, 1988b; Loftsson, 1989) on the permeation of AZP through hairless mouse skin are recorded in Table 9. Hairless mouse skin was pretreated with the penetration enhancer formulation for a duration of 1 or 12 h, prior to studying the absorption of AZP from a suspension in a buffered 10% propylene glycol vehicle. Similar pretreatment regimens have been used by other investigators (Sherertz et al., 1987; Goodman and Barry, 1988) in attempts to separate specific enhancement from partitioning, solubility and activity effects. Control pretreatments comprised the solvent upon which the enhancer solutions were based. The majority of enhancer pretreatments produced approx. 2-fold enhancement. Although the penetration enhancement reflects, to some extent, the increased skin-vehicle partitioning (Table 3), disruption of the stratum corneum lipids, the proposed mechanism of penetration enhancement for some enhancers (Walters, 1989; Francoeur et al., 1990), would also influence diffusivity which is not reflected in the latter data.

TABLE 9

*Permeation data for AZP across hairless mouse skin from suspensions in 10% PG buffer (pH 4.50) following a 1 or 12 h pretreatment with various penetration enhancers.*

| Pretreatment | Flux (mol $cm^{-2}$ $h^{-1}$) | $K_p$ (cm $h^{-1}$) | Flux ratio [c] |
|---|---|---|---|
| Azone (3%) in PG | 5.318 (0.222) | 0.257 (0.011) | 1.89 |
| Azone (3%) in TS | 4.477 (0.298) | 0.216 (0.014) | 1.18 |
| Oleic acid (5%) in PG | 7.009 (0.183) | 0.338 (0.009) | 2.49 |
| DCMS (15%) in PG | 6.171 (0.275) | 0.298 (0.013) | 2.19 |
| DCA (0.5%) in PG | 4.543 (0.479) | 0.219 (0.023) | 1.61 |
| DCA (0.5%) in PG [a] | 3.045 (0.283) | 0.147 (0.014) | 1.74 |
| PG | 2.821 (0.271) | 0.136 (0.013) | – |
| PG [a] | 1.753 (0.077) | 0.0845 (0.004) | – |
| TS [b] | 3.781 (0.267) | 0.0182 (0.013) | – |
| No pretreatment 12 h equilibration | 2.712 (0.138) | 0.131 (0.007) | |
| No pretreatment 1 h equilibration | 1.974 (0.050) | 0.0952 (0.0024) | – |

[a] 1 h pretreatment, all other pretreatments were for 12 h.
[b] 0.1% v/v Tween 20 in normal saline.
[c] Flux ratio = flux following pretreatment with enhancer/flux following pretreatment with appropriate vehicle (i.e., TS or PG).
PG, propylene glycol; TS, Tween 20 (0.1%) in normal saline; DCMS, dodecylmethyl sulphoxide; DCA, dodecylamine; values in parentheses are the standard errors on the mean.

Propylene glycol has also been reported to promote skin penetration, of for instance, metronidazole and oestradiol (Møllgaard and Hoelgaard, 1983a,b). In the current study, no such effect was observed following pretreatment of skin with propylene glycol, in comparison to penetration of AZP across skin which had been allowed to equilibrate for the same period of time without treatment. This was true for both 1 and 12 h pretreatment periods (Table 9). The evidence regarding the role of this accelerant appears to be conflicting and suggests that, in most cases, its effect is due to a modification of the thermodynamic status of the formulation through its solubilising properties (Woodford and Barry, 1986). The activity of other enhancers may, however, be significantly improved when applied in combination with propylene glycol (Cooper, 1984; Wotton et al., 1985) indicating a synergistic effect.

The lipophilic amine, dodecylamine, was also investigated for potential penetration enhancement properties. The facilitated transport of anionic drugs by ion-pairing with a series of *N*-substituted bis(2-hydroxypropyl)amines (Barker and Hadgraft, 1981) has been reported and this amine ($pK_a$ 10.63; Albert and Serjeant, 1984) was chosen to provide a counterion, which may ion-pair with dissociated AZP and thus potentially enhance its skin permeation. Inclusion of dodecylamine (DCA) in the donor formulation failed to produce flux levels higher than those with a vehicle without DCA (Table 10). In fact, the flux ratio suggests a suppression of penetration, possibly due to the lower diffusivity associated with a large counterion. Increasing the pH of the donor vehicle, which should promote ion-pairing due to the increased ionisation of AZP, also failed to increase penetration compared to that from a vehicle of lower pH. In contrast, pretreatment with DCA does enhance penetration. This suggests that barrier impairment, as opposed to ion-pairing, may be responsible for this observation. Although 12 h pretreatment periods were usually employed, either a 1 h pretreatment with 0.5% DCA in PG produced similar enhancement of AZP permeation (Table 9). Permeation experiments utilising the weak base caffeine were performed in order to separate the effects of ion-pairing from barrier compromisation. Caffeine has a $pK_a$ value of 0.6 (Florence and Attwood, 1988) and exists essentially in the unionised form at the pH under investigation (4.50). Thus, the potential for ion-pairing is eliminated. Skin was pretreated for a period of 1 h with a 0.5% w/v solution of DCA in propylene glycol prior to following the permeation of caffeine from a suspension in 10% propylene glycol in buffer. A control experiment studying the diffusion of caffeine across skin pretreated with propylene glycol alone was also conducted. The resulting permeation profiles are presented in Fig. 1. The significant and progressive penetration enhancement of caffeine following pretreatment with DCA confirms that a reduction of barrier resistance, rather than ion-pairing, is the predominant mechanism of enhancement.

TABLE 10

*Effect of dodecylamine (0.5%) on the permeation of AZP across hairless mouse skin from a suspension in 10% PG at pH 4.50*

| Treatment | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | Flux ratio |
|---|---|---|
| DCA in PG | 1.471 (0.205) | 0.75 |
| DCA in PG [a] | 1.761 (0.286) | 0.89 |
| 1 h pretreatment with DCA in PG | 3.045 (0.283) | 1.74 |
| 12 h pretreatment with DCA in PG | 4.543 (0.479) | 1.61 |
| 1 h pretreatment with PG | 1.753 (0.073) | – |
| 12 h pretreatment with PG | 2.821 (0.271) | – |
| No pretreatment | 1.974 (0.050) | – |
| No pretreatment [a] | 1.980 (0.093) | – |

[a] pH, 6.50.
Values in parentheses are the standard errors on the mean.

The permeation of AZP from donor suspensions of various pH values was also investigated following 12 h pretreatment with 3% Azone in propylene glycol and permeation data are summarised in Table 11. These results demonstrate an increase in flux as pH increases, with only a weak fall in the magnitude of the permeability coefficient. The observations are in contrast to the influence of pH without enhancer treatment (Table 7), where flux was independent of pH and

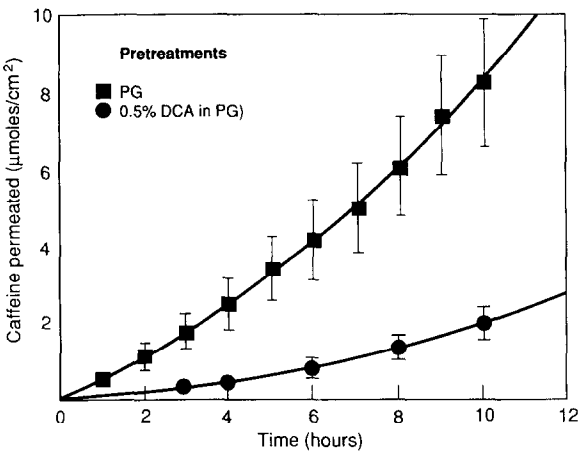


Fig. 1. Permeation profile for caffeine across hairless mouse skin with and without pretreatment (1 h) with dodecylamine (0.5%) in propylene glycol. The donor phase was a suspension in aqueous propylene glycol (10%, pH 4.50). (●) Pretreatment with propylene glycol; (■) pretreatment with dodecylamine (0.5%) in propylene glycol.

$K_p$ fell markedly. These data suggest that penetration is favoured by the ionised species, possibly as a result of an ion-pairing mechanism (Hadgraft, 1985). It has been suggested that Azone may be capable of forming ion-pairs with anionic drugs, based on the observed promotion of salicylate transport across an artificial lipid membrane in the presence of Azone. However, such models may also be explained in terms of partition alone (Irwin and Smith, 1991). It should be noted that a pH gradient, to provide a driving force for ion-pair transport, was not employed in the current study. Enhanced penetration by the ionised species following Azone pretreatment is also unexpected in view of the partitioning data where the major effect is on $P_u$ (Table 3). These observations are not consistent with enhancement by ion-pairing and other influences may be important. These include the interaction of vehicle pH, Azone and propylene glycol. This solvent is known to penetrate the stratum corneum (Polano and Ponec, 1976; Møllgaard and Hoelgaard, 1983a,b), a process promoted by Azone (Wotton et al., 1985), and a modified solubilising capacity for the permeant within the site (Barry, 1987) may result which has not been adequately modelled by equilibrium partition data.

TABLE 11

*Permeation data for AZP across hairless mouse skin from suspensions in buffered 10% PG (pH 4.50–6.50) following 12 h pretreatment with 3% Azone in PG*

| Donor pH | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | $K_p$ (cm h$^{-1}$) |
|---|---|---|
| 4.50 | 5.318 (0.222) | 0.257 (0.011) |
| 5.00 | 6.696 (0.447) | 0.227 (0.015) |
| 5.50 | 7.271 (0.311) | 0.186 (0.008) |
| 6.00 | 7.872 (0.371) | 0.165 |
| 6.50 | 10.23 (1.18) | 0.187 (0.022) |

Values in parentheses are the standard errors on the mean.

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