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J. Pharm. Pharmacol. 1985, 37: 725–727
Communicated April 9, 1985

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Facilitated transport of sodium salicylate across an artificial lipid membrane by Azone

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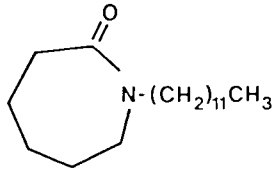
The ability of Azone (1-dodecylazacylcoheptan-2-one), a recently developed penetration enhancer, to facilitate the transport of sodium salicylate across an artificial lipid membrane has been investigated using the rotating diffusion cell, a well defined model for percutaneous absorption. Azone was found to be capable of enhancing the transport of the salicylate anion across an isopropyl myristate membrane, by using a pH gradient as the chemical driving force. The results indicate that Azone may be capable of forming ion pairs with anionic drugs.

Azone (I) is a comparatively new compound that has been shown to enhance the percutaneous penetration of many compounds (Stoughton & McClure 1983). Its exact mechanism of action is unknown. Previous work

(I) 

in our laboratory has shown that long chain tertiary amines are capable of facilitating the transport of anionic drug molecules across artificial lipid membranes, by using a pH gradient to provide the driving force (Barker & Hadgraft 1981). The surface of the skin is reported to be slightly acidic, pH 4·2–5·6 and the lower layers are at the physiological pH of 7·4 (Katz & Poulsen 1971). We have therefore employed a pH gradient of 5–7·4 in our model system to represent the natural pH gradient that exists in skin.

The facilitated transport scheme, shown in Fig. 1, is established in the rotating diffusion cell. The epidermal barrier is simulated by a membrane filter impregnated with isopropyl myristate (IPM), a liquid representative of skin lipids (Poulsen et al 1968). In our previous experiments the carrier was incorporated into the IPM membrane. At the lower pH of the donor compartment/membrane interface the carrier protonates and can combine with the anions present to form ion pairs, in the interfacial region. The ion pairs can then partition into the bulk lipid phase and diffuse down their concentration gradient to the opposite interface. In the interfacial region at the higher pH the carrier deprotonates to release the anions.

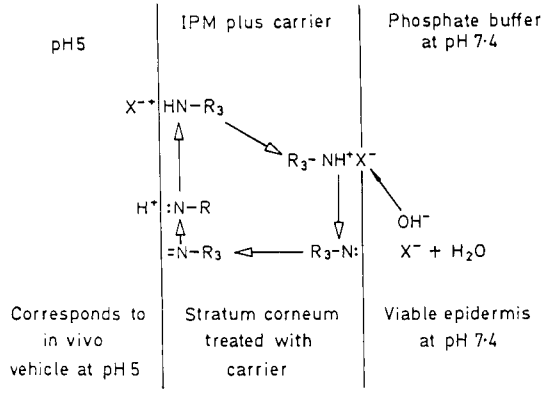


FIG. 1. Proposed facilitated transport scheme.

Azone has been reported to form the hydrobromide salt when treated with anhydrous hydrogen bromide (Stoughton & McClure 1983) and the same authors also suggest that Azone may be capable of forming salts with strong acids. Since Azone has a nitrogen atom in its ring structure which can be protonated it was therefore thought to be capable of operating within such a facilitated transfer scheme, although the pK_a of this

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nitrogen group is not known (it would be difficult to determine accurately a meaningful pK_a for a compound that is essentially water insoluble). The results obtained from this system are reported here.

Materials and methods

Sodium salicylate (BDH), isopropyl myristate 98% (IPM, Sigma Chemical Co.), dimethyldichlorosilane 2% in 1,1,1-trichloroethane (BDH) were used as received. Azone (1-dodecylazacycloheptan-2-one) was a gift from Nelson Research Ltd, Ethomeen S12 (*N,N*-bis(2-hydroxyethyl)oleylamine) was a gift from Akzo Chemie U.K. Ltd. Both were used without further purification. Cellulose nitrate 1 μm pore size membrane filters were from Whatman Ltd.

Partitioning experiments. Extraction coefficients of salicylate between aqueous solutions and IPM or 0·1 M Azone dissolved in IPM, were obtained by using a filter probe technique (Tomlinson 1982) over a pH range of 2·5–7·5 using a citric acid/phosphate buffer according to the formula of McIlvaine. Salicylate content in the aqueous phase was monitored in a flow through system, by uv spectrophotometry, at 298 nm. Measurements were carried out at 32 °C using a thermostatted beaker. The aqueous and organic phases had been presaturated with one another by equilibration overnight before the experiments.

Rotating diffusion cell studies. The rotating diffusion cell (RDC) was used to study the transfer of the salicylate anion across a lipoidal membrane. This cell uses the hydrodynamics of the rotating disc to impose a known pattern of convective flow on either side of the membrane (Albery et al 1976). The membrane consists of a cellulose nitrate membrane filter which is first rendered hydrophobic by treatment with a solution of 1,2-dimethyldichlorosilane 2% in 1,1,1-trichloroethane. The IPM (or a solution of carrier in IPM) is then applied dropwise to the membrane. Any excess lipid phase is then carefully removed with a soft tissue. In previous work this has been shown to be a reproducible method of producing such membranes. In all experiments described here the pH gradients were maintained by using a pH stat technique to keep the donor compartment at pH 5, whilst the receptor compartment consisted of Sörensen's phosphate buffer, pH 7·4. The volumes of the donor and receptor compartments were 250 ml and 30 ml, respectively. The rate of appearance of salicylate in the receptor phase was monitored continuously using a flow through cell in a uv spectrophotometer at 298 nm. Experiments were carried out at 32 °C, maintained using a thermostatted glass jacket surrounding the RDC. The apparent rate constants were determined at least six times for each rotation speed, using at least three separate membranes, and a lag period of at least 1 h was allowed before any flux measurements were determined.

Results and discussion

The flux (J) of salicylate across the membrane in the RDC is related to a first order rate constant $\vec{k}$ by $J = \vec{k}AC$, where A is the effective area of the membrane and C is the concentration of the salicylate in the donor compartment. The method of analysing data from the RDC is to plot the inverse forward rate constant (calculated from the flux of solute across the membrane) as a function of the inverse square root of the rotation speed of the cell. The forward rate constant depends on the flux of anion across the membrane, the interfaces and the stagnant diffusion layers on either side of the membrane. From this type of analysis it is possible to determine the forward rate constant at infinite rotation speed, i.e. with no stagnant diffusion layers present on either side of the membrane. The results from the rotating cell are presented in this manner in Fig. 2. The gradients reflect the diffusion of the salicylate in the aqueous stagnant diffusion layers on either side of the IPM-impregnated membrane. From the intercepts it can be seen that 0·1 M Azone in IPM has increased the flux of salicylate across the membrane by a factor of 1·7. We have previously found Ethomeen S12 to be a very potent carrier amine (Hadgraft et al 1984), and in a concentration of 0·025 M within the membrane, it increases the flux of salicylate across the membrane by a factor of approximately 3. These results are included in Fig. 2 for comparison.

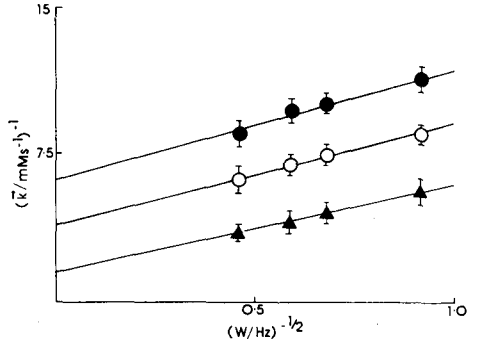

FIG. 2. Relation between the inverse forward rate constant ($\vec{k}$) and the inverse square root of rotation speed for sodium salicylate across an isopropyl myristate membrane (●), 0·1 M Azone membrane (○) and a 0·025 M Ethomeen S12 membrane (▲).

The results obtained suggest that Azone increases the flux of salicylate across the membrane by forming ion pairs with the salicylate. At the concentration used it is unlikely that Azone is acting as a cosolvent, and it is thought that the ring nitrogen can protonate sufficiently at pH 5 to participate in an ion pair extraction process.

To determine whether Azone is capable of forming ion pairs with the salicylate anion, or whether it acts as a co-solvent in enhancing the transport of salicylate across a lipid membrane we investigated the extraction of the salicylate anion from aqueous solution into the organic

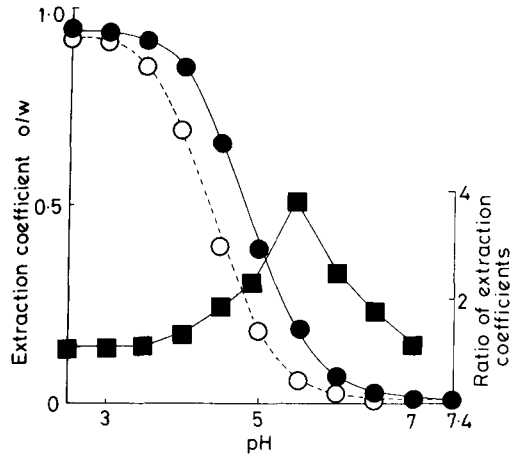

FIG. 3. pH extraction profiles for salicylate between aqueous solution and IPM (○), and 0·1 M Azone in IPM (●). The relation between the extraction coefficient into 0·1 M Azone in IPM, divided by the extraction coefficient into IPM is also shown (■).

phase, with or without Azone present, as a function of the pH of the aqueous phase.

The relation between the extraction coefficients of salicylate between aqueous solutions and IPM, or 0·1 M Azone in IPM over the pH range investigated is shown in Fig. 3. At low pH values the salicylate will be present in its un-ionized form and consequently efficient extraction of salicylate into the organic phase takes place. At high pH values salicylate is present in its ionized form and the extraction becomes less efficient. The converse will be true of the ionization state of Azone at the oil/water interface, which will be fully ionized at low pH, and only partially ionized at high pH. The pH/extraction profile for salicylate between the aqueous and oil phases, shows a shift to the right in the presence of 0·1 M Azone in the IPM. The extraction of salicylate by the Azone becomes more significant as the pH increases, i.e. as the proportion of ionized salicylate available for ion pairing increases. This in turn is countered by a reduction in the amount of ionized Azone available for ion pairing as the pH increases, and explains why the extraction of salicylate in the presence of Azone also becomes less efficient at higher pH value. This is illustrated by the plot of the extraction coefficient of salicylate into IPM plus 0·1 M Azone divided by the extraction coefficient into IPM (Fig. 3) which produces a bell shaped curve, reaching a maximum at pH 5·5, where we propose the optimum conditions for ion pair formation between Azone and salicylate in this system may occur. Such behaviour can be explained on the basis of an ion pairing mechanism. If Azone were acting as a cosolvent it would remove salicylate at a constant level and is unlikely to be affected by changes in the aqueous pH as seen here.

These findings may have important implications for any future topical drug delivery systems containing Azone as a penetration enhancer for anionic drug molecules. Ionized molecules do not readily penetrate the stratum corneum and Azone may be used to effect transfer of anionic entities. It is also possible in two phase topical formulations for the Azone to ion pair with anionic species present and hold them in the lipid phase. This would alter the thermodynamic activity of the anionic drug and consequently affect its bioavailability.

We thank SERC and Fisons Pharmaceuticals for a CASE award for P. K. W.

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