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Mechanism of action of Azone as a percutaneous penetration enhancer: lipid bilayer fluidity and transition temperature effects

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Summary

Azone is an effective penetration enhancer for the percutaneous delivery of certain topically applied drugs. Fundamental physicochemical experiments have been performed to elucidate the mechanism of action of Azone. The penetration enhancing effect of Azone is believed to be due to its increasing the fluidity of the intercellular lipid bilayers of the stratum corneum. Phospholipid vesicles were chosen as a simple model to represent these bilayers. The effect of Azone on phase transition temperature and lipid fluidity was studied using turbidity and fluorescent probe (pyrene excimer) technique. Addition of increasing amounts of Azone to the bilayer resulted in lowering of phase transition temperature, shown by turbidity of vesicle suspensions, and an increase in lipid fluidity, shown by changes in pyrene fluorescence. The results suggest that Azone would interact with stratum corneum lipids in a similar manner, thereby reducing the diffusional resistance of the stratum corneum to drugs with balanced hydrophilic–lipophilic characteristics.

Introduction

Recent developments in transdermal drug delivery have brought about a renewed interest in the mechanisms of percutaneous absorption. In order to extend the range of drugs which can be administered via the skin and also to increase the efficiency of locally acting drugs it is necessary to include penetration enhancers in the formulations. The mechanism of action of penetration enhancers is not clearly understood. However, if these can be identified it should be possible to rationalise and optimise their structural design.

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The stratum corneum provides the principal barrier to the percutaneous penetration of topically applied substances. For a few materials which are extremely lipophilic, the primary resistance may be transferred to the underlying viable aqueous tissues. It is therefore important to determine the route of penetration through the stratum corneum.

Until about a decade ago it was considered that the intercellular channels occupied such a small relative surface area that they were an insignificant route. However, Albery and Hadgraft (1979) demonstrated that the route of penetration of esters of nicotinic acid was via intercellular channels. At that time the composition of the intercellular space had not been clarified. Recently Williams and Elias (1987) have studied their lipid nature and have demonstrated that the stratum

corneum is composed of lipid-depleted keratinised cells embedded in a matrix consisting of predominantly neutral lipids, arranged in structured bilayers. Swartzendruber et al. (1987) have suggested that the corneocytes are surrounded by a lipid envelope, although it is unclear how this may contribute as a route for percutaneous penetration. Studies of percutaneous absorption through solvent-treated skin (Bucks et al., 1983) and in essential fatty acid-deficient mammals (Elias et al., 1980) have supported this theory, since in both cases a disturbance of barrier function occurs as a result of disruption of the intercellular lipids. The intercellular route has thus been revealed as a potential avenue of percutaneous transport for relatively non-polar drugs with partially lipophilic nature.

Azone is a novel percutaneous penetration enhancer which effectively promotes the absorption of certain drugs (Stoughton and McClure, 1983). Much research has been performed on the effect of Azone on the transport of specific drugs across the skin (Chow et al., 1984; Rajadhyaksha et al., 1985; Wotton et al., 1985; Hosoya et al., 1987; Sheth et al., 1986) but very little work has been concerned with elucidation of the mechanism of action of Azone. The fluidity of the membrane lipids have been shown to be of great importance in determining the permeability characteristics of a membrane (Chapman et al., 1986). Using differential scanning calorimetry, Azone has been shown to fluidise the lipids of the stratum corneum (Goodman and Barry, 1985).

We have studied the effect of Azone on lipid fluidity using a liposome model for the structured intercellular lipid bilayers of the stratum corneum. Initial experiments on DSC of human stratum corneum (Beastall and Hadgraft, to be published) demonstrated the presence of a melting transition in the range (27–41°C), and so multilamellar vesicles prepared from L-α-dipalmitoylphosphatidylcholine (DPPC), with a melting transition at 41.8°C, were chosen as a simple reproducible model system. The effect of Azone on the gel/liquid crystal transition temperature (T_m) of the lipid was studied by measuring the turbidity of the liposomal suspension as a function of temperature; the suspension undergoes a sharp decrease in turbidity at T_m. The fluidity of the lipid was studied using the lipophilic fluorescent probe, pyrene. The use of the pyrene excimer method of measuring membrane fluidity has been described previously (Galla and Sackmann, 1974; Azzi, 1975; Galla and Hartmann, 1980).

Materials and Methods

Materials

DPPC and pyrene were obtained from Sigma and used without further purification. Azone was a gift from Nelson Research, Irvine, CA.

Preparation of multilamellar vesicles (MLV) containing Azone

Hand-shaken MLV were prepared according to the method of Bangham et al. (1965). The lipid (10 mg) and the desired quantity of Azone were dissolved in chloroform in a 50 ml round-bottomed flask; the chloroform was removed on a rotary evaporator to leave a thin film of lipid. 10 ml of deionized water was added and the sample vortexed above T_m at 50°C. The suspension was incubated above T_m for a further 2 h with intermittent vortexing, and left overnight at 4°C prior to use to ensure complete hydration of the lipid.

Preparation of small unilamellar vesicles (SUV) containing pyrene and Azone

The vesicles were prepared as described above, but pyrene was added to the chloroform solution prior to evaporation to produce a pyrene concentration of 50 mmol per mole of lipid (4.75 mol%). After incubation, the lipid suspensions were sonicated above T_m for 20 min using a 1-min on–off cycle, until the suspension became a clear homogeneous dispersion. This produced SUV, which were also allowed to stand overnight at 4°C to hydrate fully. It was necessary to employ SUV for fluorescence experiments to avoid sedimentation of the sample during the experiment.

Determination of T_m

MLV suspension optical density was recorded as a function of temperature using a white-light absorptiometer constructed by the authors. This

allowed the sample to be heated via a water recirculator and stirred using a magnetic flea. The solution temperature was monitored continuously using a 591 kH temperature sensor (RS components), and temperature/absorbance data was continuously recorded for analysis using an Acorn BBC model B computer. The water recirculator raised the sample temperature at rates of 5–10 °C/min. The transition temperature of the lipid was measured in the same way as used for DSC, i.e. the temperature at which a tangent to the transition intersected the baseline.

Fluorescence spectroscopy

Fluorescence measurements were performed with a Perkin-Elmer 3000 spectrofluorimeter. Pyrene was excited at 335 nm, and the emissions of monomer and excimer were measured at their maxima of 396 and 470 nm, respectively.

Samples (SUV) were held in a 1-cm quartz cuvette in a thermostatted cell-holder, which was heated via a water recirculator (Grant Instruments). Spectra were recorded at 2 °C intervals. The sample temperature was measured directly using a digital thermometer.

Results

Fig. 1 shows the optical density of DPPC MLV, containing 0, 33 and 70 mol% Azone, as a function of temperature. Liposomes containing no Azone melted sharply (within 1–2 °C) at 41 ± 1 °C, in agreement with the literature value of 41.4 °C (Mabrey and Sturtevant, 1976). Addition of Azone decreased the onset temperature, broadened the transition, and abolished the pretransition.

The value of T_m as a function of Azone concentration in the MLV is shown in Fig. 2. T_m decreases in an apparently linear fashion ($r = 0.996$) in liposomes containing up to 60 mol% Azone, which had a T_m of 34.5 °C. The pretransition is not detectable above an Azone concentration of 33 mol%.

The fluorescence data is shown in Fig. 3 as the excimer/monomer emission intensity ratio as a function of temperature. The control SUV containing no Azone showed a slight decrease in

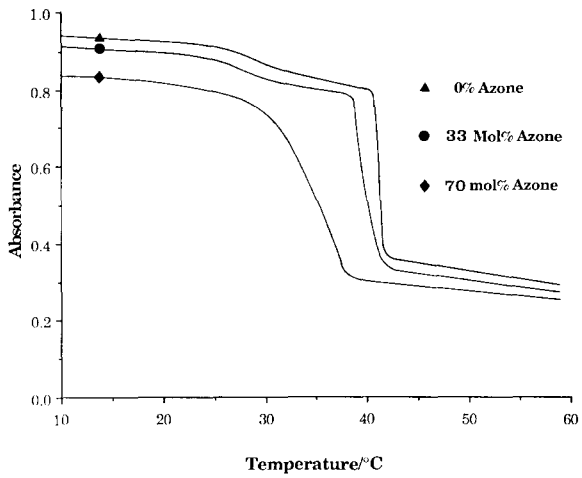

Fig. 1. Absorbance–temperature profiles for DPPC MLV containing Azone.

excimer emission at the pretransition temperature, followed by an increase in emission to a maximum at 42 °C, with a decrease with further temperature increases. Addition of Azone had three significant effects.

(1) Variations in excimer emission intensity in the pretransition region were abolished.
(2) The peak at T_m shifted to progressively lower temperatures with increasing pyrene concentrations.
(3) The excimer emission intensity below T_m increased with increasing pyrene concentration.

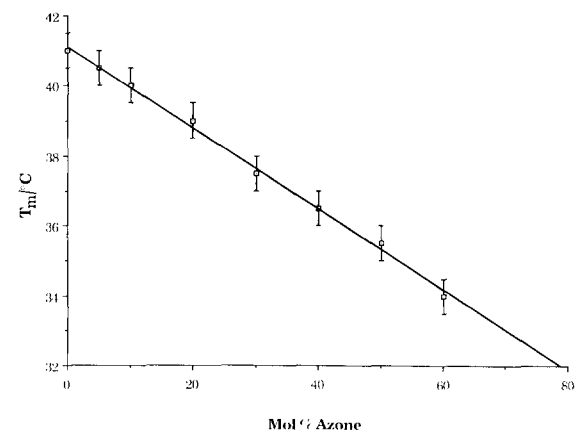

Fig. 2. Transition temperature of DPPC MLV as a function of added Azone concentration, measured by optical absorbance of liposomal suspensions.

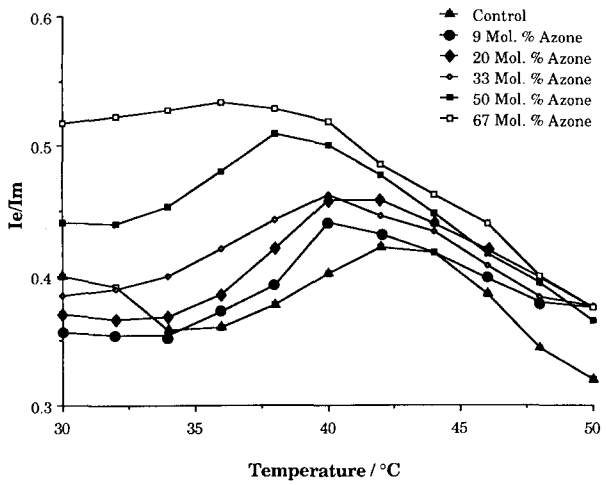

Fig. 3. Pyrene excimer/monomer emission ratios as a function of temperature and Azone concentration in DPPC MLV.

By contrast, differences in excimer emission with Azone content above T_m are less marked, although still present.

The temperature of maximum excimer emission is plotted in Fig. 4 as a function of Azone concentration. This temperature decreases monotonically with increasing Azone concentration.

Discussion

The incorporation of Azone into lipid bilayers decreased the transition temperature. This is similar to the effects of a number of other molecules such as anaesthetics (Lee, 1976; Vanderkooi et al., 1977), alcohols (Rowe, 1982) and penetration enhancers such as dimethyl sulphoxide (Surewicz, 1984). Since the transition temperatures observed in skin lipids are near 35 and 70 °C (Rehfeld and Elias, 1982), it is likely that partitioning of topically applied Azone could reduce the transition temperature by several degrees to a temperature below body temperature, producing a more permeable liquid crystal layer.

Phase transitions in lipids are cooperative phenomena; that is, the behaviour of a particular molecule in one phase is dependent upon the state of the other molecules around it. The sharpness of the transition depends on the number of molecules forced to cooperate in the transition (Jain and Wagner, 1980). The broadening of the phase transition on addition of Azone to the membrane suggests that Azone intercalates into the bilayer and affects the number of other lipid molecules that a single lipid molecule may influence. The more Azone present within the membrane, the greater the reduction in the size of the cooperative unit.

Numerous compounds have been used as membrane probes, the most useful being ANS (Azzi, 1975), parinaric acid (Sklar, 1977a and b) and pyrene (Galla and Sackmann, 1974). Pyrene excimer formation is a second-order process, the rate

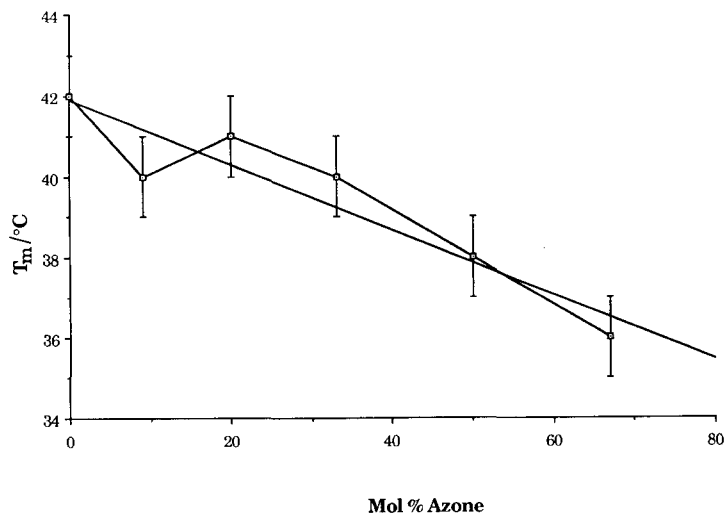

Fig. 4. Transition temperature of DPPC MLV as a function of added Azone concentration, measured by pyrene excimer emission.

of which is dependent on competition between excited monomer decay and collision with a ground-state species to form the excimer. The ratio of excimer to monomer emission, I_e/I_m is given by:

$$\frac{I_e}{I_m} = kcD$$

where D is the pyrene diffusion coefficient in the lipid membrane, c the concentration of pyrene, and k a lumped constant involving factors such as fluorescence lifetimes and quantum yields, which do not vary in a given system. Hence excimer emission is favoured by high diffusion coefficient (laterally within the membrane) and high local pyrene concentration.

Galla and Sackmann (1974) have studied pyrene excimer formation in model membranes, and have concluded that pyrene is not appreciably soluble in gel-phase lipid membranes. This results in the separation of pyrene into local clusters within the membrane, the so-called phenomenon of phase separation. Evidence for this is provided by a decrease in excimer emission on melting of the lipid bilayer; if pyrene were present as a 2-dimensional isotropic solution, the increase in diffusion coefficient on melting should result in increased excimer formation. Pyrene molecules in clusters are thought to be aligned parallel to one another, enhancing excimer formation below T_m. Above T_m the pyrene clusters disperse.

As the temperature of the lipid/pyrene mixture was increased from a point below T_m, mobility of the pyrene molecules increased, both within a cluster and between clusters; consequently the excimer yield increased in accord with theory. At T_m the pyrene clusters began to disperse, hence the local concentration of pyrene molecules decreased and excimer emission fell, despite the assumed increase in lateral probe mobility at T_m. The fact that excimer emission continued to fall up to 12 °C above T_m suggests that excimer formation was dominated by dispersal of pyrene clusters over the temperature range from T_m to 50 °C.

Increasing concentrations of Azone caused increased excimer emission below T_m, reflecting the increased lipid fluidity. This effect is most marked at high Azone concentrations. Additionally T_m, as marked by the temperature at which excimer emission began to decrease, is shifted to lower temperatures, in agreement with the transition temperature measurements by turbidity. The transition temperatures measured by the excimer technique are consistently higher than those measured by optical density of liposome suspensions; this may partly be due to the difficulty in locating the temperature of maximum excimer emission, and partly since the pyrene molecule will to some extent interact with Azone, particularly at high Azone concentrations.

There is an additional source of systematic error in measurements of lipid mobilities by pyrene probes. Many authors make no mention of corrections for polarization of emission. Instrument polarization is usually disregarded as a constant effect; however, it can cause significant differences in intensities in the pyrene system, particularly where large changes in mobility and membrane order occur. The changes in the polarization of emission of several probes is in fact the basis of another widely used method of measuring membrane fluidity; diphenylhexatriene is a notable example (Shinitzky and Barenholz, 1978). Changes in polarization may contribute to the apparent decrease in excimer emission above T_m. Additionally, polarization effects may be the cause of differences seen in pyrene probe behaviour by workers using different spectrofluorimeters with different polarization characteristics.

Conclusions

The percutaneous penetration enhancer Azone has been shown to exert a considerable effect on the fluidity of lipid model membranes, and consequently is expected to have a similar effect on intercellular lipid channels in vivo. Both fluorescent probe and liposome melting studies confirm that Azone decreases T_m in lipid bilayers sufficiently to induce formation of a liquid phase, and increases membrane fluidity below T_m. These observations are fully consistent with its observed actions as a penetration enhancer.

Penetration enhancers may act by altering the diffusion characteristics of the skin or by modifying the partitioning behaviour of the drug at the stratum corneum–viable epidermal interface. The former is more significant for drugs with balanced hydrophilic–lipophilic characteristics. The latter will be dominant for drugs that are lipophilic. The probable mechanism of action of Azone is to interact with the structured lipids in the intercellular channels. This will render them more fluid and decrease the diffusional resistance in this region. Azone is thus more likely to affect the transfer of drugs with balanced hydrophilic–lipophilic characteristics, since the reduced diffusional resistance so produced makes this a more favourable route than the transcellular pathway. This is in agreement with published work on the enhancing properties of Azone (Stoughton and McClure, 1983). If this type of enhancer is required to promote the penetration of lipophilic drugs it will have to be formulated with a co-solvent whose role is to modify stratum corneum–viable tissue partitioning. Such synergy has been previously observed, e.g. mixtures of Azone and propylene glycol are effective in promoting the absorption of metronidazole (Wotton et al., 1985; Sheth et al., 1986).

In order to optimise the design of novel formulations rationally, it is important to understand the precise mechanism of action of penetration enhancers. Basic physicochemical studies as detailed above are important in elucidating the fundamental processes operating in these systems.

Acknowledgements

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