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Vehicle effect on topical drug delivery. III. Effect of Azone on the cutaneous permeation of metronidazole and propylene glycol

Paul K. Wotton [2], Birgitte Møllgaard [1], Jonathan Hadgraft [2] and Annie Hoelgaard [1]

[1] *Department of Pharmaceutics, Royal Danish School of Pharmacy, DK-2100 Copenhagen (Denmark) and* [2] *Department of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD (U.K.)*

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Summary

The ability of Azone (1-dodecylazacycloheptan-2-one), a novel penetration enhancer, to increase the percutaneous delivery of metronidazole has been investigated, across full thickness human skin in vitro. A finite dose technique was used employing several vehicles. Azone at a concentration of 1% was found to be as effective as 5% and 10% concentrations in achieving enhanced transport of metronidazole. The presence of propylene glycol was found to be necessary for maximal enhancement, and the penetration of this compound was also found to be markedly enhanced by Azone. The delivery of metronidazole within the first 20 h of the experiments was increased about 25 times in the presence of 1% Azone. Repeated doses of the drug after a single dose of Azone indicated that the effect of Azone on the skin remains after several days.

Introduction

The skin, or more strictly the stratum corneum provides an efficient barrier to the diffusion of most drugs and only a few drug molecules have the optimal physicochemical properties to penetrate it sufficiently to be therapeutically efficacious. One method of overcoming this problem is to incorporate solvents into the vehicle that increase the thermodynamic activity of the drug in the vehicle so as to promote transfer of the drug into the stratum corneum.

Correspondence: A. Hoelgaard, Department of Pharmaceutics, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

Another approach is the inclusion of solvents that actually affect the barrier function of the stratum corneum. Dimethyl sulphoxide (DMSO) is the most widely studied of these. However, DMSO has the disadvantage that it needs to be present in high concentrations in order to produce a significant enhancement, and at these concentrations it is irritant to human skin. Azone (1-dodecylazacycloheptan-2-one) is a comparatively new compound that has been reported as being an enhancer of percutaneous penetration (Stoughton, 1982), although its mechanism of action is unknown. It has been shown to enhance the percutaneous penetration of a number of hydrophobic and hydrophilic compounds, even when present in concentrations as low as 1% (Stoughton and McClure, 1983). Azone is also claimed to be non-irritant to human skin, even when applied undiluted (Stoughton, 1982). Most in vitro studies involving Azone have been carried out using hairless mouse skin, in this work the effects of Azone on the permeation of metronidazole have been studied using full thickness human skin in vitro.

Materials and Methods

Chemicals

Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) [1] (Fig. 1) was used as the model drug since its permeation characteristics have been well determined in

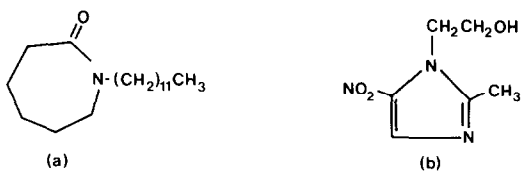

Fig. 1. Chemical structure of Azone (a) and metronidazole (b).

TABLE 1

VEHICLES USED FOR PREPARATION OF TEST SOLUTIONS

Vehicle	Ethanol solutions of:		
	Azone % v/v	PG % w/v	PEG 400 % w/v
A	–	18	–
B	1	18	–
C	5	18	–
D	10	18	–
E	1	–	–
F	1	–	18

[1] Dumex Ltd., Denmark

this laboratory. Vehicles used were propylene glycol (PG) [2] and polyethylene glycol 400 (PEG 400) [3]. Azone was a gift from Nelson Research. Its chemical structure is given in Fig. 1.

Preparation of test solutions

The test solutions were prepared from the vehicles given in Table 1. Metronidazole was dissolved in the vehicles to produce test solutions of 18 μmol metronidazole/ml.

Permeation procedure

Open glass diffusion cells having an available diffusion area of 1.8 cm^2 were employed. Fresh mamma operation tissue was separated from the subcutaneous fat and stored at $-18°C$ for a period not exceeding 2 weeks. All experiments were performed on skin from the same donor. The details of the operation of the diffusion cells were as described previously (Møllgaard and Hoelgaard, 1983a). The permeation studies of each vehicle were conducted at least in duplicate, the data obtained being reproducible within $\pm 20\%$ from the donor, a figure within that normally expected from this type of experimental procedure (Southwell et al., 1984).

100 μl of the test solution was applied to the surface of the skin. A dose of 1 $\mu mol \cdot cm^{-2}$ metronidazole was thus applied.

Analysis

Concentrations of metronidazole and propylene glycol in the receptor phase were determined using an HPLC method and a GC method, respectively.

Concentrations of metronidazole were measured by a Pye Unicam Model PU 4020 variable wavelength detector with a Waters model 6000A pump. Sample injection was made by complete filling of a 20-μl loop injection valve. The column, 25 cm long and 4.0 mm i.d., was packed with Lichrosorb RP-8 (7 μm particle size). The reverse-phase column was eluted, at ambient temperature with a mobile phase consisting of 0.05 M acetate buffer pH 4.5–methanol (55 : 45). The flow rate was 1 $ml \cdot min^{-1}$. The column effluent was monitored at 320 nm. Under these conditions metronidazole showed a retention time of 3.6 min.

Concentrations of PG were measured by a Pye Unicam Gas Chromatograph equipped with a FI detector. The glass column, 3 ft. $\times$ 4 mm i.d., was packed with Porepak P 80/100 mesh and operated at 200°C with the nitrogen flow rate at 45 $ml \cdot min^{-1}$. Samples of 5 μl were injected. Under these conditions PG showed a retention time of 2.4 min.

Results

Propylene glycol was chosen as the main vehicle since its use has been well defined in this laboratory. PG has also been shown to permeate the skin together

[2] Ph. Eur., III.
[3] Ph. Nord. 63, II.

with metronidazole in previous studies (Møllgaard and Hoelgaard, 1983c). Therefore it was pertinent to investigate whether Azone would affect the permeation of the vehicle as well as the drug.

The effect of including 1% Azone in the vehicle (vehicle B) on the permeation of metronidazole through human skin is shown in Fig. 2. It can be seen clearly that Azone has considerably enhanced the permeation of metronidazole with a concurrent reduction in the lag time for permeation. After 20 h in the presence of 1% Azone, 81% of the metronidazole dose had been delivered across the skin whereas without Azone present only 3% was delivered. This represents an enhanced delivery by more than 25 times. The inclusion of 1% Azone in the vehicle reduced the lag time for permeation from approximately 22 h to about 5 h.

It is also interesting to notice the effect Azone has on the permeation of PG through the skin. From Fig. 2 it is clear that 1% Azone has an enhancing effect, the permeation profiles showing a similar time course to those of metronidazole. Møllgaard and Hoelgaard (1983c) found a correlation between metronidazole and PG permeation and suggested that the ability of PG to decrease the diffusional resistance of the skin barrier might be due to the drug being dissolved in PG whilst it permeates the stratum corneum. The results seen here would seem to support this theory.

In order to determine how long the effect of Azone lasted on human skin in vitro, a multidose experiment was carried out. The test solution containing 1% Azone (vehicle B) was applied to the skin as the first dose, subsequent applications of

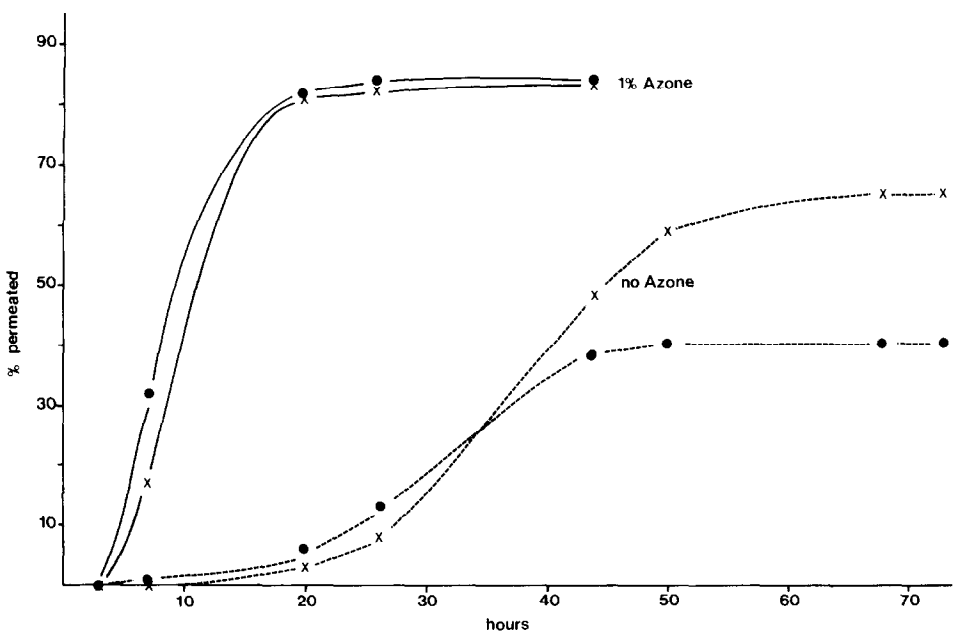

Fig. 2. Permeation of metronidazole (×) and propylene glycol (●) from vehicle A with no Azone present (- - - - - -) and vehicle B containing 1% Azone (————) through human skin in vitro.

metronidazole were made using vehicle A, i.e. a vehicle containing no Azone. As a control an experiment was run using vehicle A for dosing throughout. The results of the multidose experiments are shown in Fig. 3. The graphs illustrate that the permeation-enhancing effect of Azone remains at least over a period of 120 h after a single application.

The optimal concentration of Azone is known to vary with both the drug and the formulation. In general, however, concentrations of 2–10% appear to be appropriate to most formulations (Stoughton and McClure, 1983). Therefore it is of interest to see whether Azone concentrations in the range of 1–10% affect the permeability of the skin to metronidazole and propylene glycol. The efficiency of the vehicle containing varying amounts of Azone is illustrated in Fig. 4. The 20-h permeation of metronidazole and PG was found to be nearly independent of the Azone concentration in the range investigated. About 70–80% of the dose of both metronidazole and PG permeated during the first 20 h.

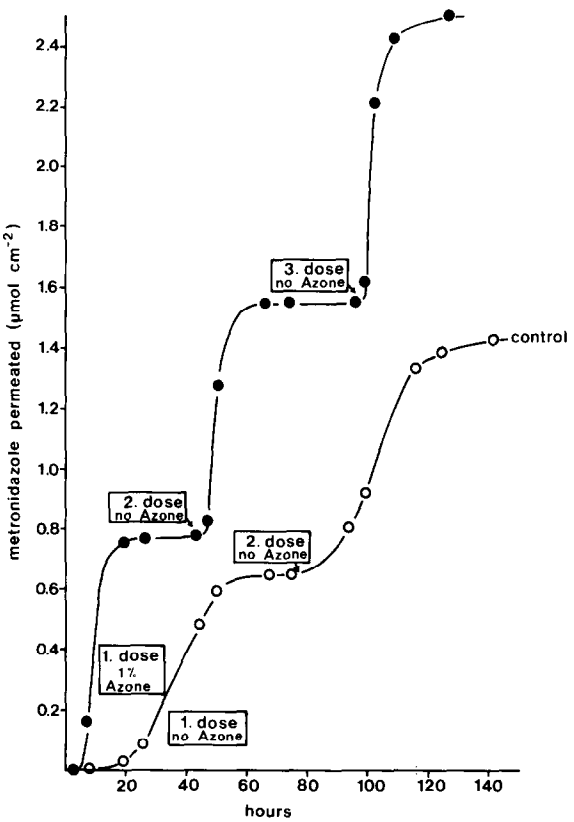

Fig. 3. Multidose permeation of metronidazole through human skin in vitro. All doses applied were 1 μmol·cm^{-2}. ●, first dose was applied in vehicle B containing 1% Azone and subsequent doses were applied in vehicle A containing no Azone; ○, control where both doses were applied in vehicle A.

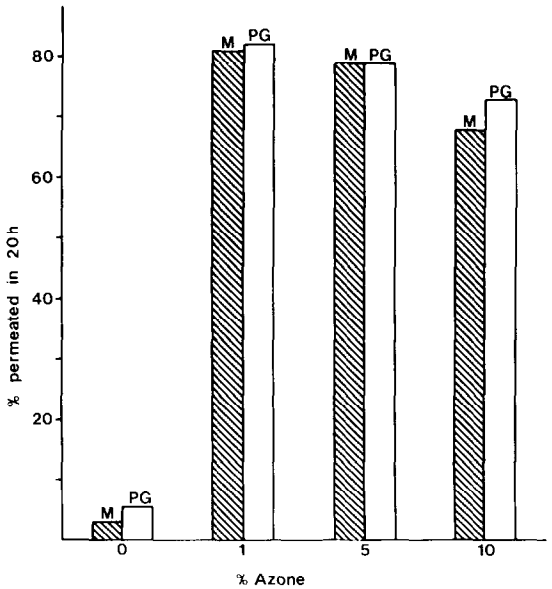

Fig. 4. 20-h permeation of metronidazole (M) and propylene glycol (PG) through human skin in vitro, from vehicles containing various amount of Azone (vehicles A–D listed in Table 1).

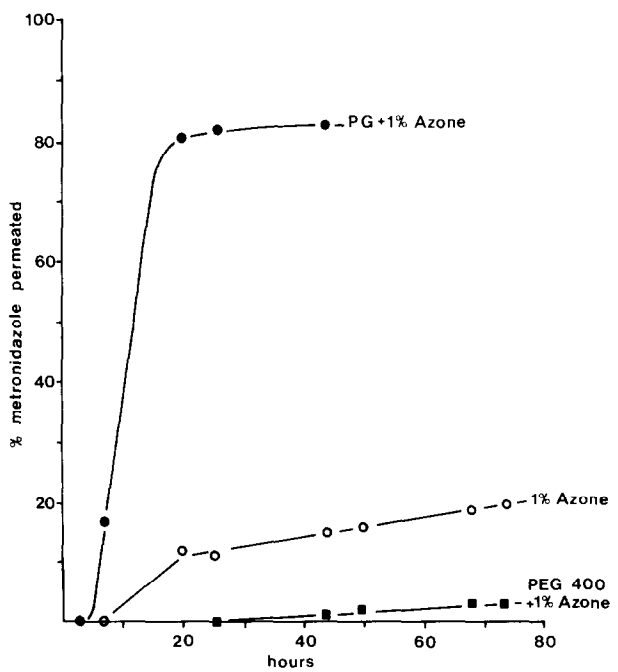

Fig. 5. Permeation of metronidazole from vehicle B containing 1% Azone + 18% PG (●), vehicle E containing 1% Azone (○) and vehicle F containing 1% Azone + 18% PEG 400 (■) through human skin in vitro.

In order to see whether the effect of Azone in some way is related to its coexistence with PG in the vehicles, experiments without PG and with an alternative glycol, PEG 400 were performed. PEG 400 was chosen because it is shown not to improve metronidazole permeation (Møllgaard and Hoelgaard, 1983b) and not to permeate the skin in detectable amounts [4]. Omission of propylene glycol from the vehicle (vehicle E) reduced the permeation of metronidazole as shown, but replacement of PG with PEG 400 (vehicle F) produced an extremely slow permeation of metronidazole, presumably a result of the drug not being released from the vehicle that does not penetrate the skin itself. Thus the choice of vehicle is an important factor; a penetration enhancer that acts on the skin itself like Azone can only function if the drug is made available to the skin by the vehicle.

Discussion

The results presented here show that Azone markedly increased the cutaneous permeation of metronidazole, with a significant reduction in the lag time when PG was used in the vehicle. Comparison of the total dose of metronidazole delivered after the plateau level had been reached, was similar in the presence and absence of Azone (Fig. 2), which is expected when using a finite dose technique. The time taken to reach the plateau is considerably reduced in the presence of Azone. The fact that Azone enhanced the permeation of PG in the same manner as the drug permeation is enhanced indicates that the two components are transported through the same microenvironment of the skin, the microenvironment where Azone is effective. PG is a vehicle constituent in many topical formulations and it might be undesirable that the skin is made more permeable to this vehicle excipient.

The multidose experiment showed that a single application of Azone is capable of enhancing the permeation of subsequent doses of drug, under the conditions of our experiments, for a period of at least 5 days. These in vitro results suggest that Azone may disrupt the rate-limiting barrier for a period of several days. However, from the experimental technique used it is not clear how much Azone remains on the skin surface after the initial dose. In vivo this prolonged activity might still be seen but may be modified by various metabolic processes. Azone should therefore be capable of enhancing the permeation of most drugs when applied under favourable conditions since it appears to act on the skin membrane itself, rather than acting as a solvent for the drug. When a drug is applied under less ideal conditions, as illustrated by the results obtained when PEG 400 was employed as vehicle, the activity of Azone can be inhibited. Therefore, if Azone is to be used as an accelerator the final formulation will be a particularly important factor to consider.

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[4] GC method used: the column, 3 ft. × 2 mm, was packed with Tenax G.C, 60/80 mesh, and operated at 350°C.

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