International Journal of Pharmaceutics, 43 (1988) 233–240
Elsevier

IJP 01471

Vehicle effect on topical drug delivery. IV. Effect of *N*-methylpyrrolidone and polar lipids on percutaneous drug transport

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(Received 27 October 1987)
(Accepted 16 November 1987)

Key words: Skin permeability; Permeation enhancer; Vehicle effect; Metronidazole; Polar lipid; *N*-Methyl-2-pyrrolidone; Excised skin

Summary

The ability of *N*-methylpyrrolidone and polar lipids to increase the percutaneous delivery of metronidazole was investigated across full thickness human non-occluded skin in vitro. A finite dose technique was used employing several vehicles. Fatty acids and alcohol were effective in presence of propylene glycol, whereas *N*-methylpyrrolidone increased metronidazole penetration in presence of isopropyl myristate. Moreover, *N*-methylpyrrolidone was found to permeate the skin readily when applied in the neat state or in a mixture with isopropyl myristate. The combined results indicate that the variation in barrier permeability to metronidazole is associated with the rate of *N*-methylpyrrolidone permeating the skin.

Introduction

Dermal therapy is generally restricted to a limited number of drugs according to the main function of skin as a protective barrier towards entering compounds. One efficient method to circumvent the barrier function of the skin is to include in a formulation, a penetration enhancer, which temporarily increases the permeability of the stratum corneum (for review, see Barry, 1987; Cooper and Berner, 1987).

The stratum corneum is structurally a complex membrane, and thus permeation of drugs probably takes place through different pathways. In addition, there is strong evidence that penetration enhancers act on different permeation pathways to varying degrees (Barry and Bennett, 1987). Morphological and biochemical studies (Elias, 1983) support the physical–chemical evidence of separate hydrophilic and lipophilic domains in the barrier area and penetrants with both hydrophilic and lipophilic properties probably penetrate the stratum corneum most readily. Thus to examine fundamental mechanisms of various penetration enhancers, it is reasonable to select a model drug with intermediate polarity, e.g. metronidazole.

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The purpose of the present study is to assess the effect of various penetration enhancers on the permeation of metronidazole. Solvents which are expected to mainly affect the polar domains, e.g. *N*-methylpyrrolidone (Barry and Bennett, 1987), or the non-polar domains, e.g. fatty acids and alcohols (Cooper, 1984; Cooper et al., 1985) are included. Further the change in barrier capacity of the excised human skin following its occlusive hydration was quantified by use of metronidazole as tracer.

Materials and Methods

Chemicals

Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) (Dumex, Denmark) was used as the model drug. The vehicles used were: *N*-methyl-2-pyrrolidone (NMP) (Merck), linoleic acid (LA) (Sigma), oleic acid (OA) (Sigma), oleic alcohol (OAl) (Sigma), propylene glycol (PG) (Ph. Eur. III), isopropyl myristate (IPM) (Fluka AG).

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.

Skin membranes

Mamma operative skin was used in all experiments except in one study, i.e. measuring permeation of NMP (Fig. 3), where female abdominal cadaver skin was used. The skin was separated from subcutanous fat and stored at $-18\,^{\circ}C$ until time of use. In the studies with epidermis the full thickness skin was separated by being exposed to $60\,^{\circ}C$ hot water for 6 min. In the experiments with delipidized skin, the skin mounted in the diffusion cells was treated with chloroform methanol (2 : 1) mixture in the following manner: 2 ml organic solvent was left on the surface for 5 min and exchanged with fresh solvent 4 times.

Permeation studies

The diffusion cell assembly and the experimental procedures for the permeation studies were as previously described (Møllgaard and Hoelgaard, 1983a). The epidermal side of the skin was exposed to ambient laboratory atmosphere except for some experiments, sealed off airtight in order

TABLE 1

Vehicles used for preparation of test solutions

Vehicle	Ethanol solutions of:					
	NMP %v/v	LA %v/v	OA %v/v	OAl %v/v	PG %w/v	IPM %w/v
A	–	–	–	–	18	–
B	5	–	–	–	–	–
C	5	–	–	–	18	–
D	5	–	–	–	–	18
E	10	–	–	–	–	–
F	10	–	–	–	18	–
G	10	–	–	–	–	18
H	–	1	–	–	18	–
I	–	5	–	–	18	–
J	–	10	–	–	18	–
K	–	–	1	–	18	–
L	–	–	5	–	18	–
M	–	–	10	–	18	–
N	–	–	–	1	18	–
O	–	–	–	5	18	–
P	–	–	–	10	18	–

to simulate occlusion of the skin. The dermal side was in contact with the recipient phase (7.5 ml), which consisted of 0.05 M isotonic phosphate buffer pH 7.4. Before the test solution was applied, the skin was allowed to equilibrate in the cells overnight. Test solutions were applied to the skin surface in the amount of 56 $\mu l/cm^2$. A dose of 1 $\mu mol/cm^2$ metronidazole was thus applied.

Analysis

Concentrations of metronidazole and NMP in the recipient phase were determined using HPLC methods. Concentration of metronidazole was measured by a Hitachi model 655A variable wavelength UV detector with a model 655A-11 pump and a Hitachi D-2000 Chromato-Integrator. Sample injection was made by complete filling of a 20 μl loop injection valve. The column, 25 cm long and 4 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 4.0 min.

Concentrations of NMP were measured by a Cecil CE 2012 UV monitor with a Kontron LC pump T414. Sample injection was made by complete filling of a 100 μl loop injection valve. The column, 25 cm long and 4 mm i.d., was packed with Techopak C18 (10 μm particle size). The reverse-phase column was eluted at ambient temperature with a mobile phase consisting of water/methanol (85 : 15). The flow rate was 2 $ml \cdot min^{-1}$. The column effluent was monitored at 195 nm. Under these conditions NMP showed a retention time of 4.4 min.

Results

Effect of occlusion

Several reports indicate that hydration of the skin by occlusion increases the skin permeability to various substances (Blank, 1985). Since hydration can influence diffusivity, partition coefficient and thickness, the effect of hydration cannot always be predicted. The effect of hydration of full thickness skin on metronidazole permeation is shown in Fig. 1. It can be seen that hydration provides a pronounced increase in skin permeability to metronidazole. Epidermis alone and full thickness delipidized skin were also included in order to see whether or not these membranes were altered by hydration to the same extent as full thickness skin. Fig. 2 shows the percentage metronidazole transported after 24 h through the 3 types of skin membranes. Drug transport and effect of hydration were essentially the same in the full thickness skin and in the epidermis. However, the barrier capacity of the skin diminished by its delipidization through extraction of the fatty materials. Mixtures of water-miscible and water-immiscible organic solvents have shown to extract efficiently skin lipids (Scheuplein and Ross, 1970). Since skin lipids are a major constituent of the stratum corneum barrier, the permeability becomes too high for an effect of hydration on drug transport to be observed.

It is possible that the marked effect of occlusive hydration of full thickness skin obscures an enhancing ability of an added solvent. Therefore, the subsequent studies on vehicle effect were made by use of non-occluded excised skin membranes.

Effect of NMP

The effect of including 5% NMP in two commonly used solvents in topical formulations, e.g. PG and IPM, is shown in Table 2. Metronidazole in PG-vehicle (vehicle A) was used as a reference system of comparison as its use has been well defined in this laboratory (Møllgaard and Hoelgaard, 1983b and c). It appeared that the 23 h permeation was not affected by including NMP in the PG-vehicle whereas NMP alone and in mixture with IPM promoted drug permeation. The NMP/IPM vehicle (vehicle D) had delivered about 4 times as much metronidazole as the PG-vehicles (vehicle A and C) during 23 h. The difference in drug permeation from vehicles with NMP/IPM and NMP/PG cannot be due to difference in the thermodynamic activity as the drug solubility in the vehicles is almost similar (Table 2).

In order to see whether or not the effect of NMP in some way is related to its concurrent

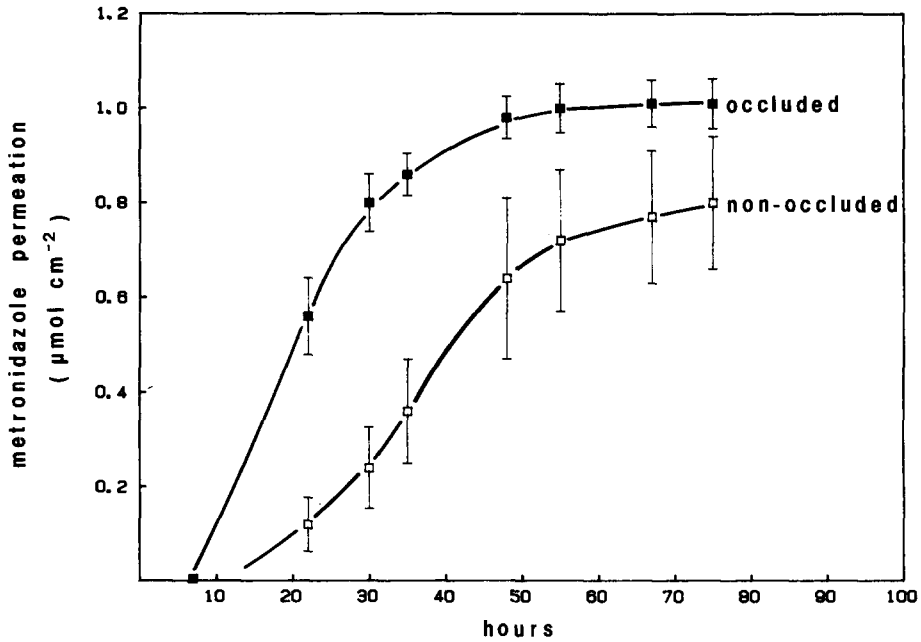

Fig. 1. Effect of occlusion on permeation of metronidazole through human skin in vitro (vehicle A).

permeation of the skin, the permeation profiles of NMP were determined. In Fig. 3 is shown the permeation profiles of NMP applied on the skin in the neat state or in mixtures with IPM and PG, respectively. NMP passed readily across the skin and NMP permeation showed a similar pattern as metronidazole delivery. Omission of PG from the vehicle increased permeation of NMP as shown, and replacement of PG with IPM produced an extremely fast permeation of NMP, presumably a result of the substance being easily released from the vehicle.

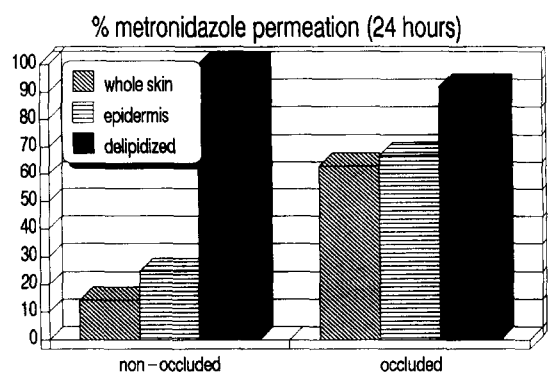

Fig. 2. Effect of occlusion on permeation of metronidazole through different types of skin membranes (vehicle A).

Effect of polar lipids

Two component systems consisting of a hydrophilic molecule such as PG and a lipophilic molecule such as fatty acids or alcohols have shown to be very effective permeation promoters (Cooper, 1984; Cooper et al., 1985). Fig. 4 illustrates the transport rate of metronidazole from vehicles containing an increasing percentage of polar lipids up

TABLE 2

Comparison of 23 h permeation of metronidazole from various vehicles

Vehicle [1]	Enhancement ratio [2]	Solubility of metronidazole (mg·g^{-1}) [3]
A (PG)	1.0	18
B (NMP)	2.7	187
C (NMP/PG)	0.95	22
D (NMP/IPM)	3.8	25

[1] See Table 1.
[2] Relative to vehicle A.
[3] In the vehicle without ethanol.

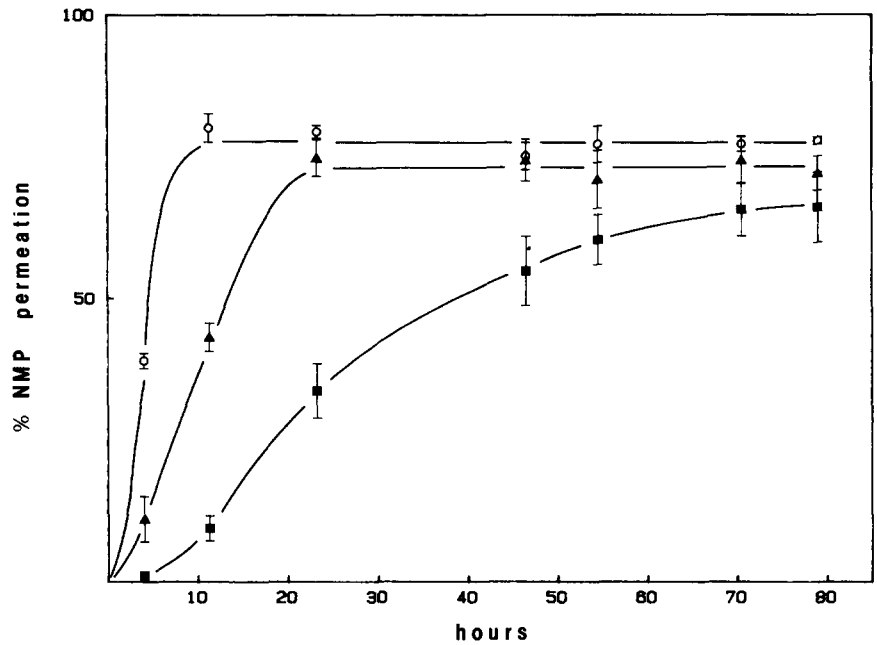

Fig. 3. Permeation of *N*-methylpyrrolidone (NMP) through human skin in vitro after application of 3 vehicles. Key: ○, NMP in IPM (vehicle G); ▲, NMP neat (vehicle E); ■, NMP in PG (vehicle F).

to 10% in PG-vehicles. The results show that all 3 polar lipids produce enhanced drug permeation (note that comparisons are not directly possible because of varying permeation hours). Addition of a small amount of oleic alcohol to PG-vehicle provides for a rather large increase in the metronidazole transport. As the concentration of oleic acid or linoleic acid is increased, the drug transport is also increased up to a point. The use of a mixture of the two materials, PG and e.g. 10% LA gives a 30-fold increase in transport rate.

It has been suggested that these binary systems

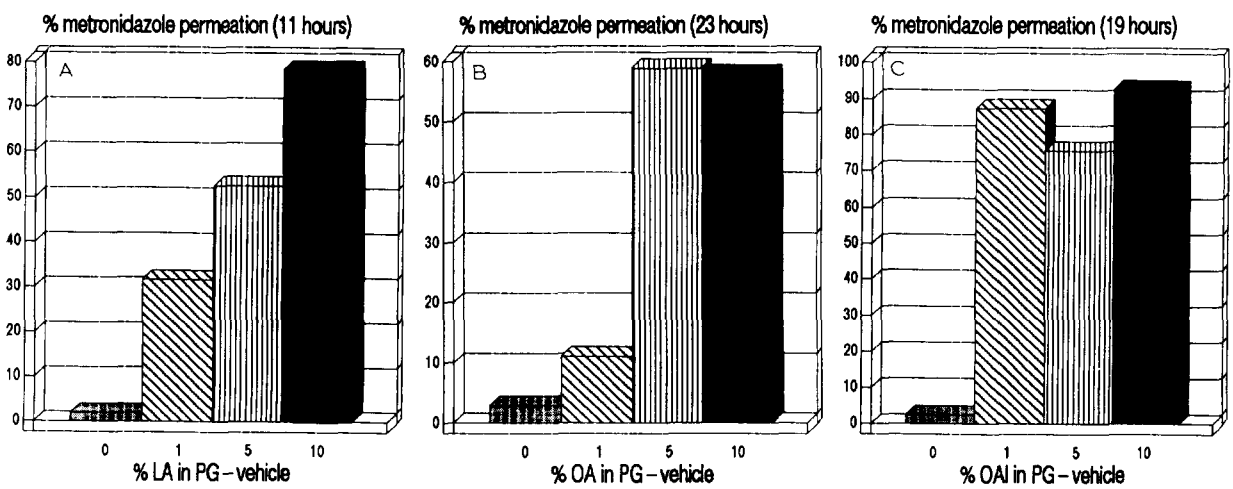

Fig. 4. Effect of addition of polar lipids on the permeation of metronidazole in propylene glycol vehicle across human skin in vitro. Key: LA, linoleic acid (vehicle H, I and J), OA, oleic acid (vehicle K, L and M), OAl, oleic alcohol (vehicle N, O and P).

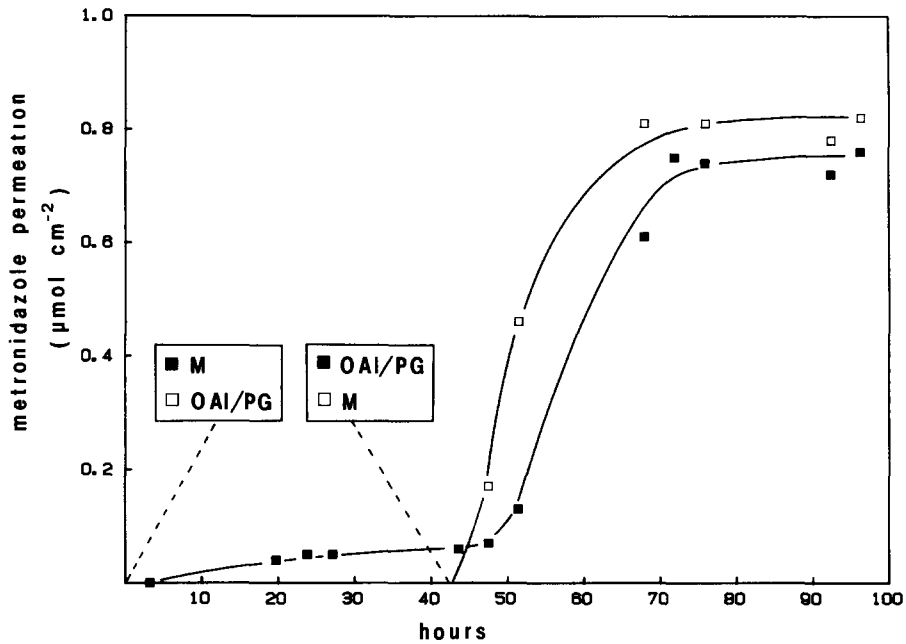

Fig. 5. Effect of time lag between application of oleic alcohol–propylene glycol (vehicle N) and metronidazole on the permeation of metronidazole through human skin in vitro. Key: ■, metronidazole applied as deposited film at $t = 0$ h and vehicle N applied at 42 h; vehicle N applied at $t = 0$ h and metronidazole applied as deposited film at $t = 42$ h.

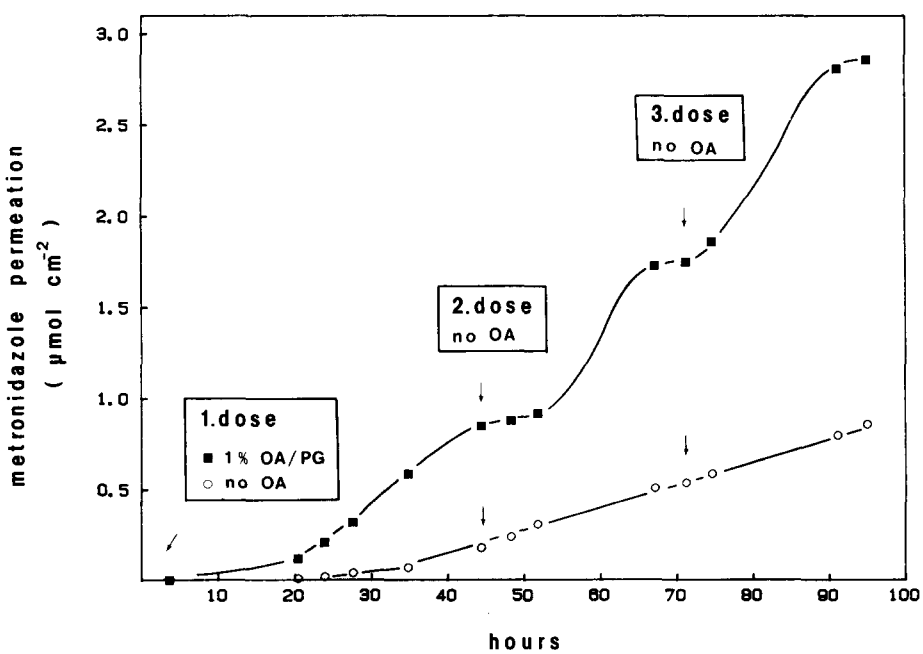

Fig. 6. Multidose permeation of metronidazole through human skin in vitro. All doses applied were 1 μmol · cm^{-2}. Key: ■, first dose was applied in vehicle K containing 1% oleic acid and subsequent doses were applied in vehicle A containing no oleic acid; ○, control where all 3 doses were applied in vehicle A.

are capable of disorganizing the multilaminate hydrophilic–lipophilic layers located intercellularly in stratum corneum. The ability of vehicles to perturb the barrier can be evaluated in time lag application studies. For example, the ability of the OAl/PG-vehicle (vehicle N) to affect the barrier in vitro is demonstrated in Fig. 5. Pretreatment with this vehicle for 42 h before drug application (in ethanol solution) enhanced the metronidazole permeation in the same manner as application in the opposite order. Thus the effect of this vehicle cannot merely be due to some specific drug–vehicle interaction, but must indeed involve a direct influence on the barrier structure.

In order to determine how long the effect of these binary systems lasted on human skin in vitro, a multidose experiment was carried out. The test solution containing OA/PG (vehicle K) was applied to the skin as the first dose, subsequent applications of metronidazole were made using vehicle A, i.e. a vehicle containing no fatty acid. As a control an experiment was run using vehicle A for dosing throughout. The results of the multidose experiments are shown in Fig. 6. The graphs illustrate that the permeation-enhancing effect of OA/PG vehicle remains at least over a period of 95 h after a single application. However, at this point it is important to emphasize that in vitro studies may not provide reliable insight into the ability of an accellerator system to sustain its effect in vivo.

Discussion

It is often stated that there is no universal vehicle for all drugs. Thus each topical system must by designed around the drug it contains to optimize the therapeutic effect of the active ingredient. The results presented here show that the effect of the penetration enhancer is highly dependent on the cosolvent in the vehicle. Thus PG is an effective vehicle component in combination with polar lipids in delivering metronidazole through the skin whereas the penetration enhancer, NMP, is not effective in the presence of PG. As it appears, NMP is not readily being released from the PG-vehicle to the skin presumably because of some specific accelerant–vehicle interactions. On the contrary, IPM is efficient for delivering both NMP and metronidazole across the skin. IPM is a hydrophobic solvent unlike PG of which the hydroxyl groups possibly can associate with, for instance, NMP by hydrogen bonding. Consequently, the escaping tendency of NMP is expected to be greater from IPM than from PG and even from neat NMP.

From the time lag studies it is evident that a single application of polar lipids in binary mixtures with PG is capable of enhancing the permeation of a drug for a period of up to 2 days, and the results derived from multidose studies show that the effect of these vehicles is not reversible within the first 95 h after application. These in vitro results suggest that the effect is not merely a question of acting as a solvent for the drug, but the enhancing system may moreover disrupt the rate-limiting barrier for a longer period of time.

Acknowledgement

This work was supported by the Danish Medical Research Council.

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