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EFFECTS OF SOME HYDROPHILIC PERMEATION ENHANCERS ON THE ABSORPTION OF BEPRIDIL THROUGH EXCISED HUMAN SKIN

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ABSTRACT

The permeation of Bepridil through excised human skin was measured from vehicles composed of various mixtures of aqueous buffer and the permeation enhancers ethanol, DMSO or DMF. Only DMSO was found to act as a true permeation enhancer for the drug, the magnitude of its action depending on its concentration in the vehicle. At concentrations greater than 50% DMSO the permeability coefficient of the drug was increased over and above that which could be accounted for by changes

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in partitioning of the drug betweeen vehicle and skin. The effects of ethanol could be related to changes in the measured skin/vehicle partition coefficients of the drug. DMF showed a complicated, concentration dependent influence on permeation.

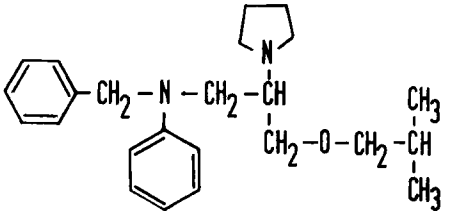
INTRODUCTION

Many drugs diffuse through human skin too slowly to produce plasma concentrations sufficient to induce satisfactory systemic effects. Various substances have been identified that increase the percutaneous absorption of certain of these drugs. Some probably act as cosolvents for the drug, allowing an increase in its activity within the vehicle. Others appear to produce some structural changes in the skin tissues, thereby altering the skin's resistance to drug permeation. All show some degree of concentration dependence, although this varies from one to the other. Azone, for example, produces enhancing effects when as little as 1% is included in the vehicle [1]. In contrast, dimethyl sulfoxide must be present in amounts exceeding 50% before being effective as an enhancer [2]. Such comparisons may, however, be spurious, since data from the literature refer to experiments performed on different drugs and using a few selected concentrations of

enhancer. A closer examination of the concentration dependence of some enhancers using a single drug would be preferable. To this end we examined the permeation of a model drug through excised human skin from aqueous vehicles containing various amounts of one of three substances identified in the literature as being permeation enhancers. Ethanol, dimethyl sulfoxide and dimethyl formamide where chosen as representitive, hydrophilic permeation enhancers which could be mixed in all proportions with water. Bepridil was used as a model drug; although not a front line candidate for transdermal drug delivery, it shows moderate skin permeability from aqueous solution and has suitable aqueous solubility. Simultaneous alterations in the drug's skin/vehicle partition coefficient caused by the presence of the enhancer in the vehicle were also determined. The amounts of drug present in the skin during steady-state permeation could thereby be compared with both permeation and partitioning data.

MATERIALS AND METHODS

Ethanol, dimethyl sulfoxide [$(CH_3)_2S=O$], and dimethyl formamide [$(CH_3)_2N-COH$] were all used as received (Fischer Scientific, Fairlawn, NJ). Bepridil (McNeil Pharmaceuticals, Spring House, PA; Lot# 8204348) was used without recrystallization:



Aqueous buffers were of the citric acid/disodium phosfate type, of ionic strength 0.1-0.2 M. Human stratum corneum plus attached epidermis (SCE) was prepared by heat separation from whole skin excised from the mid-line chest of cadavers [3]. It was stored in the dried state [4] and reconstituted by immersion for one hour in the appropriate vehicle before being used in a permeation study.

Permeation rates of the drug through SCE were measured at 37°C ± 0.5°C using twin-chambered diffusion cells made from Plexiglas and of standard design. The donor chamber was filled with a *ca.* 4 mg ml^{-1} solution of the drug in the particular vehicle under consideration, and the receiving chamber with blank vehicle. Thus the complete system, including SCE, was for the duration of the experiment as far as possible in the same milieu. Three replicate cells were run for each experiment. 20μl samples were removed from the receiving chambers at regular intervals and their Bepridil contents determined by HPLC analysis. Plots of the cumulative amount of drug permeated per cm^2 of SCE versus time were drawn, and the permeability

coefficient, **P**, determined according to:

$$\mathbf{P}\ [\text{cm h}^{-1}] = J/\Delta C$$

where J is the pseudo steady-state flux in μg cm^{-2} h^{-1} and ΔC is the concentration difference of the drug across the SCE membrane at time zero in μg cm^{-3}.

We first measured permeation at three different pHs in aqueous buffer. Mixtures of buffer and each of the permeation enhancers were then prepared covering the complete concentration range of 0 to 100% enhancer. Each of these mixtures was used as a vehicle to measure the permeation of the drug through SCE, as described above. Each permeation experiment was terminated during the pseudo steady-state phase and the drug present in the SCE tissues extracted. The SCE sample was first rinsed for a few seconds with distilled water and the drug extracted with with six, separate washings in 100 μl of methanol, each lasting for 24 h. The amount of drug present in each extract was then determined by HPLC analysis and the total mass extracted from the SCE calculated. This was then expressed as a percentage, $\%m_{ext}$, of the mass of drug originally incorporated into the donor solution.

The distribution coefficient (D) of the drug betweeen SCE and each of the vehicles examined was determined as described before [5]. D is defined here

TABLE 1

Effects of pH on the Permeation of Bepridil Through Excised Human Skin.

pH	Permeability coefficient **P** [cm $h^{-1}x10^{-4}$]	Drug extracted ($\%m_{ext}$)
4	5.90 ± 1.49	5.5 ± 0.3
5	10.8 ± 1.5	8.0 ± 2.6
6	20.7 ± 14	41.7 ± 5.4
7	34.3 ± 11.2	25.7 ± 7.8

at equilibrium as:

$$D = \frac{\mu g \text{ drug per g dry SCE}}{\mu g \text{ drug per g vehicle}}$$

Three replicate determinations were made for each vehicle.

RESULTS AND DISCUSSION

As is usual for a basic drug, Table 1 shows that **P** increases with increasing pH. This is due to the increasing proportion of undissociated drug molecules present within the aqueous vehicle which have greater permeabilities than the corresponding dissociated molecules [6]. Table 1 also indicates that greater amounts of drug (i.e. $\%m_{ext}$) tend to partition into the SCE with increasing pH. This arises as a result of the

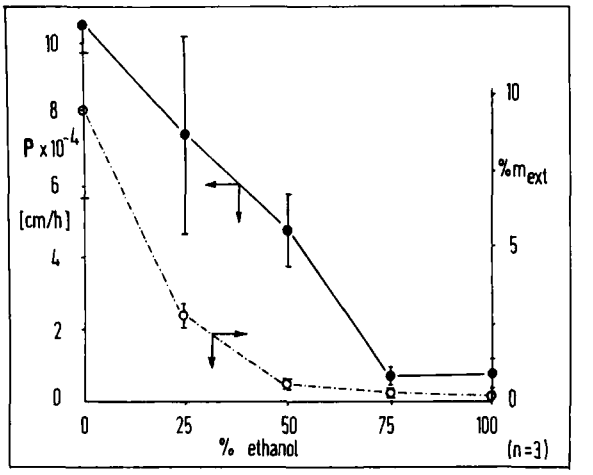


FIGURE 1

Influence of ethanol on permeability (P) and extracted drug ($\%m_{ext}$).

greater affinity of the undissociated molecules for SCE compared with the dissociated ones [5]. Indeed, at pHs 6 and 7, for example, more than one quarter of the total amount of drug originally present in the donor solution had partitioned into the SC up to the end of the experiment (48 h).

Fig.1 illustrates how increasing percentage of ethanol in the vehicle progressively reduces **P**. There is a simultaneous decrease in $\%m_{ext}$, which must reflect a reduction in the concentration gradient existing across the SCE with increasing ethanol content of the vehicle. It is clear from Fick's First Law that such a reduction in the concentration gradient will lead to decreased flux and hence decreased **P**. These results are

TABLE 2

Distribution coefficients for Bepridil Between Human Skin and Various Vehicles.

Enhancer: buffer	Ethanol	DMSO	DMF
0:100	273	273	273
10:90		3945±211	1330
20:80			602±376
25:75	54.3±27	2562±1273	
30:70			377±153
38:62		653±155	
50:50	52.8±9.3	70.8±19.5	
60:40			40.1±1.91
75:25	3.3±2.7	33.9±10.9	
80:20			26.1±6.8
85:15		3.0±3.0	
90:10	8.3±3.6	5.0±1.1	
100:0	26.7±3.8	0.72	37.4

confirmed by examining the partitioning data shown in Table 2. D is sharply reduced by increasing ethanol concentration within the vehicle, reaching a minimum value at 75%. This finding corresponds well with the changes in **P** and $\%m_{ext}$ found in this concentration range of ethanol. D increases again sightly at higher ethanol concentrations, corresponding to the range where P and $\%m_{ext}$ show only minimal changes in value. It is thus evident that the influence of ethanol on permeability can be simply explained by altered drug partitioning between vehicle and SCE. For this reason ethanol does not act as a permeation enhancer for

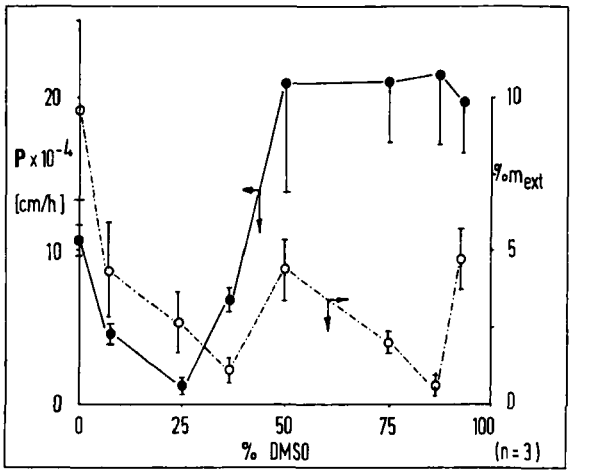


FIGURE 2

Influence of DMSO on permeability (P) and extracted drug ($\%m_{ext}$).

Bepridil but rather *decreases* **P** by virtue of reduced partitioning into the SCE. This effect on partitioning apparently outweighs any detrimental influence ethanol may have on the structure and integrity of the SCE, most particularly the stratum corneum lipids [1].

The concentration dependent action of DMSO on **P** and m_{ext} (Fig.2) is not so straightforward. With increasing DMSO concentration both **P** and $\%m_{ext}$ are at first decreased. This effect appears, therefore, to be the same as that observed with ethanol. Once more than *ca.* 25% DMSO is reached, however, **P** increases sharply, and $\%m_{ext}$ shows no definite trend. At concentrations above *ca.*50% DMSO **P** becomes twice as large as that found with the pure aqueous buffer and a true enhancing

effect is seen. This increase in **P** does not correlate with $\%m_{ext}$, which rises and falls in an erratic manner up to 90% DMSO. These changes in **P** must, therefore, result from some other influence of DMSO on the system. This conclusion is supported by the partitioning data in Table 2. D initially increases very sharply in the presence of small concentrations of DMSO, thus not corresponding to the decrease seen in both **P** and $\%m_{ext}$. The subsequent decrease in D with DMSO concentrations >25% also does not correspond to the increase in **P** now observed in this range. Thus, over the concentration range 0 - 50% DMSO there exists an apparent inverse relation between **P** and D. At higher DMSO concentrations **P** stays fairly constant despite a continual decrease in D. The effects of DMSO on **P** cannot, therefore, be readily explained in terms of altered partitioning alone. The (small) enhancing effect seen here may, therefore, arise from some alteration in the barrier properties of the SCE [7].

The effects of DMF are also strongly concentration dependent (Fig.3). There is an initial, sharp reduction in $\%m_{ext}$ upto *ca*. 25% DMF, above which it remains low over the whole range. **P** decreases at first in a parallel fashion to $\%m_{ext}$, but rises with >10% DMF, only to fall again at >60%. All **P** values are smaller than that found with the pure aqueous buffer, indicating no enhancing effect of DMF on the permeation

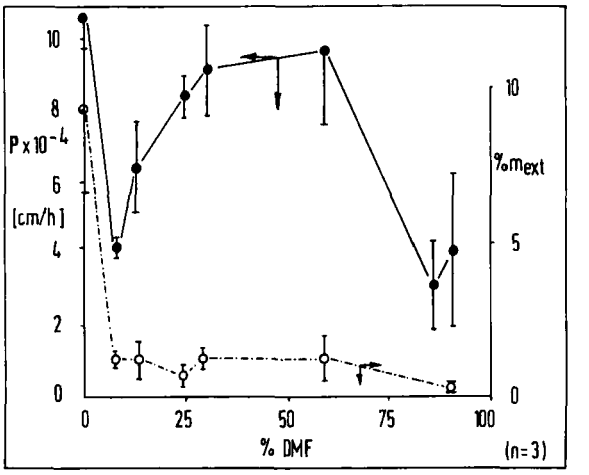


FIGURE 3

Influence of DMF on permeability (P) and extracted drug (%m_{ext}).

of Bepridil. Yet the shape of the upper curve in Fig.3 is not readily explained in terms of altered partitioning alone. The changes in D seen in Table 2 are similar to that obtained with DMSO, with D decreasing steadily after an initial sharp increase at low concentrations. Again, a sharp increase in D at low enhancer concentrations is associated with falling P and $\%m_{ext}$.

It is clear that DMF and DMSO alter the permeation of Bepridil through SC in a complicated way. The effects cannot be due solely to alteration in partitioning, as seen with ethanol. At the higher concentrations of DMSO some alteration in the barrier

properties of the SCE must occur to account for the observed effect. Although DMF does not enhance Bepridil's permeation at any of the concentrations exemined, there is also no direct relation between **P** and partitioning, as observed with ethanol.

REFERENCES

1. T. Walters, in 'Transdermal Drug Delivery', Eds., R. Hadgraft & R. Guy, Marcel Dekker, New York, 1989, p.197 et seq.
2. T. Kurihara-Bergstrom, G. Flynn and W. Higuchi, J. Pharm. Sci., **75**, 479 (1986).
3. G. Lee and P. Palicharla, Pharm. Research, **3**, 356 (1986).
4. J. Swarbrick, G. Lee and J. Brom, J. Invest. Dermatol., **78**, 63 (1982).
5. J. Swarbrick, G. Lee, J. Brom and N. Gensmantel, J. Pharm. Sci.,. **73**, 1352 (1984).
G. Lee, J. Swarbrick, J. Brom and D. Payling, Int. J. Pharmaceutics, **23**, 43 (1985).
7. B. Barry, J. Cont. Release, **6**, 85 (1987).