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Penetration enhancement in human skin; effect of 2-pyrrolidone, dimethylformamide and increased hydration on finite dose permeation of aspirin and caffeine

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Summary

This study compares the permeation of model drugs, aspirin and caffeine, under the influence of penetration enhancers 2-pyrrolidone (2-P) and dimethylformamide (DMF), with their penetration through normal and fully hydrated cadaver skin. The in vitro finite dose technique was used to mimic physiological conditions and the drugs were applied in acetone. The penetration rate versus time profile for a finite dose reached a maximum rate J_{max} at time T_{max}. For caffeine 2-P significantly increased J_{max} and the % dose penetrated in 48 h ($P = 0.031$). DMF significantly increased J_{max}, decreased T_{max} and increased the % dose penetrated in 48 h ($P = 0.031$). The aspirin data showed the same trends. However, 2-P increased the % dose of caffeine penetrated in 48 h significantly more ($P = 0.031$) than did DMF. Hydration significantly increased ($P = 0.031$) J_{max} and % dose penetrated in 48 h for caffeine. Aspirin data followed this same trend. The apparent diffusion coefficient of aspirin and caffeine through human skin increased with hydration. As hydration appears to act on skin lipids, these drugs probably penetrate, at least in part, through a lipid route in the stratum corneum.

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Introduction

Vehicles are often compared in permeation studies with drug formulations and solvents may be used to accelerate percutaneous absorption (e.g. Maibach and Feldmann, 1967; Ayres and Hooper, 1978; Windheuser et al., 1982). However, few clinical products incorporating such penetration enhancers are available, probably because their mechanisms of action and the degree of skin reversibility are not well understood, and their toxicities have not been fully established.

The action of penetration enhancers in vitro may be examined in several ways and we recently reported data from a fundamental physicochemical study which employed 2-pyrrolidone (2-P) and dimethylformamide (DMF) (Southwell and Barry, 1983). Such steady-state diffusion techniques use fully solvated skin as the control condition, although such extensive solvation or hydration of the stratum corneum is seldom strictly comparable with physiological conditions. Therefore, the present study closely mimics the in vivo situation so that in the control design, hydration and temperature gradients operate across the skin and a finite dose of drug is applied. Thus, the effects of accelerant solvents and increased moisture (obtained by occlusion) may be compared with a clinically relevant control tissue.

We chose aspirin and caffeine as our model penetrants for several reasons. They are more complex than the *n*-alcohols which we examined previously (Southwell and Barry, 1983) and hence they may possess greater potential for penetration enhancement; they are active pharmacologically and therefore of more clinical interest; they are available radiolabelled; they have been studied previously by the finite dose technique, in vitro (Franz, 1975) and in vivo (Feldmann and Maibach, 1970); and caffeine was used in our previous steady-state (infinite dose) experiments (Southwell and Barry, 1983).

Materials and Methods

Diffusion cells

Glass diffusion cells shown in Fig. 1 were used.

Preparation of full thickness skin membranes

Using a scalpel, we trimmed fat from autopsy samples of human, female, abdominal skin to provide strips of uniform thickness. We clamped these between metal plates at $-24°C$ for 1 h then warmed the upper plate to ease its removal from the stratum corneum. This procedure provided a smooth flat skin surface with the subcutaneous fat adhering to the lower plate. When the skin was warm enough to be just mobile to the touch a 430 μm thick membrane was cut using a Davies Dermatome 7 (Duplex Electro Dermatome). This membrane thus contained the entire epidermis and much of the dermis. Dermatomed skin was used immediately after preparation for diffusion experiments.

Chemicals

2-Pyrrolidone, dimethylformamide (BDH Chemicals). Acetyl [carboxy-^{14}C]salicylic acid (aspirin, Radiochemical Centre, Amersham). [1-Methyl-(^{14}C)]caffeine (New England Nuclear). Caffeine purum (Fluka agent, Fluorochem). Fisofluor-1 Scintillation Fluid (Fisons Scientific Equipment).

Control conditions

Water and temperature gradients were maintained across the skin by exposing the stratum corneum to a controlled atmosphere (22°C, 60% relative humidity) and bathing the dermal side of the skin with isotonic aqueous buffer, pH 7.4, at 37°C ± 0.5°C (water bath). The skin surface temperature was 33.0°C ± 0.4°C.

Finite dose 'in vivo mimic' method

We prepared 4 diffusion cells from each skin specimen. These were equilibrated for at least 16 h under the controlled conditions then radiolabelled drug (12–16 μg in 30 μl acetone) was applied to the stratum corneum (3.14 cm^2) as a thin film. One cell from each set provided a Control—the drug film was exposed to control conditions described above. The other cells received a penetration enhancing treatment: hydration (occlusion)—the donor compartment was covered with Parafilm; 2-P—30 μl of 2-pyrrolidone was applied; DMF—30 μl of dimethylformamide was applied.

For caffeine, 6 skin specimens were examined under all 4 treatments. For aspirin, 4 skin specimens were prepared for experiment under all 4 conditions, but some cells leaked and so only 1 specimen provided a full set of data. Statistical analysis was therefore not possible.

Receptor fluid samples (1 ml, Finpipette) were taken periodically for 100 h (volumes replaced), added to 10 ml Fisofluor-1, and counted in a Packard Tricarb Scintillation Counter, Model 3255.

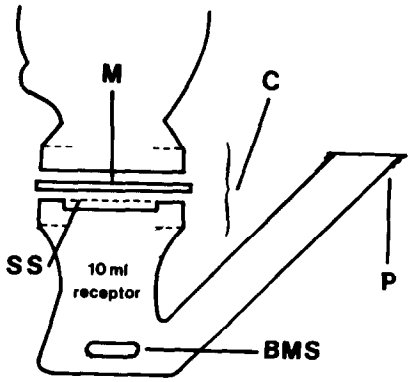

Fig. 1. Glass diffusion cell. Key: M = membrane; SS = support screen; BMS-bar magnet stirrer; P = sampling port (sealed with parafilm); C = compartments clamped across ground glass surfaces.

Theoretical considerations

If a small volume of penetrant solution is applied to the skin in a volatile solvent, a thin film of solid penetrant deposits. The penetration rate versus time plot from such a thin layer of thickness δ produces a maximum at a position described by an equation derived from the general solution of the simple membrane problem (Scheuplein and Ross, 1974).

$$\text{Time of maximum flux, } T_{max} = \frac{h^2 - \delta^2}{6D} \quad (1)$$

We can neglect δ, assumed small in comparison with h, the stratum corneum thickness. The apparent membrane diffusion coefficient is D.

For acetone-deposited films, D can be estimated from Eqn. 1. However, we cannot use this approach after the penetration-enhancing solvents have been applied, mainly because the solvents dissolve the penetrant film, the enhancers may alter dynamically the diffusional properties of the membrane as they permeate through it, and they may alter dynamically the membrane thickness (h) as they penetrate.

Results and Discussion

Example plots (Fig. 2a) illustrate data for caffeine penetrating 4 samples from one skin specimen undergoing the different treatments, in the form of cumulative percentage of the dose penetrating the membrane as a function of time. Tangents were drawn to these curves at regular time intervals to obtain the rates of penetration illustrated in Fig. 2b. From these plots, we obtain the maximum rates, J_{max}, which occur at times, T_{max}. The histograms in Fig. 3 illustrate the percentage of dose penetrated in 12 h intervals. Table 1 summarizes the results for each treatment in several skin specimens for caffeine and aspirin; the table also lists apparent mean diffusion coefficients for control and hydration treatments, derived from Eqn. 1.

Hydration, 2-P and DMF all increased the penetration of caffeine and aspirin through human skin in vitro, compared with control membranes. For caffeine penetration a statistical analysis of the data was performed using the Wilcoxon Signed Rank test. No statistical analysis was performed on the aspirin results since sufficient data points do not exist.

All treatments significantly increased J_{max}, the maximum penetration rate of caffeine ($P = 0.031$, this is the smallest possible value of P which can be obtained with 5 samples). Hydration, 2-P and DMF, all increased J_{max} for aspirin in the 4 skin specimens tested.

For caffeine, T_{max}, the time at which J_{max} occurred, was significantly earlier ($P = 0.031$) under DMF treatment than for control conditions. The aspirin data showed the same trend. In 4 out of 5 specimens 2-P provided a delayed T_{max} relative to control for caffeine, but this trend was not significant and the aspirin data did not provide the same consistent trend towards increased T_{max}.

The % dose penetrated in 48 h was significantly increased ($P = 0.031$) by all 3 treatments. However, the increase caused by 2-P was significantly greater ($P = 0.031$) than that caused by DMF.

Fig. 2 illustrates the different penetration versus time profiles for caffeine under 2-P and DMF treatment. These data suggest that DMF penetrated the stratum

Fig. 2.

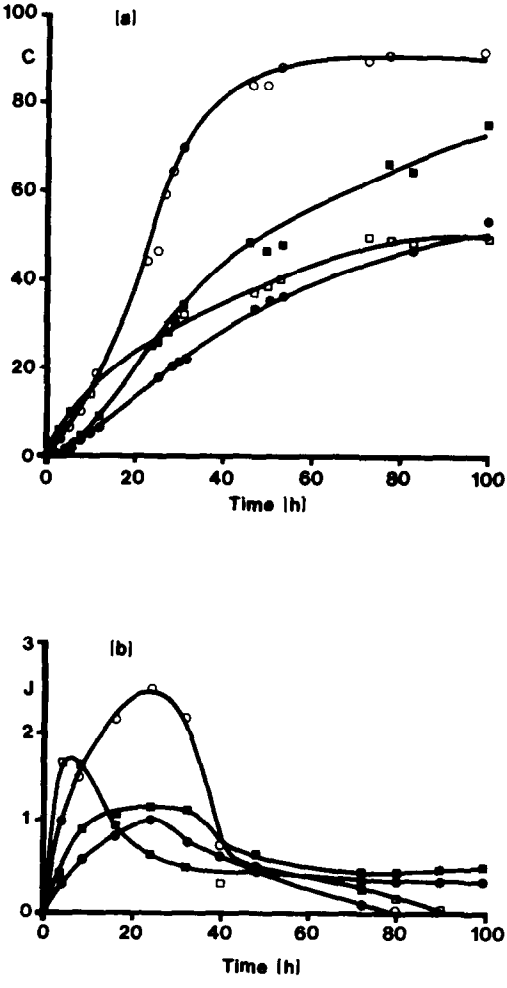

Fig. 3.

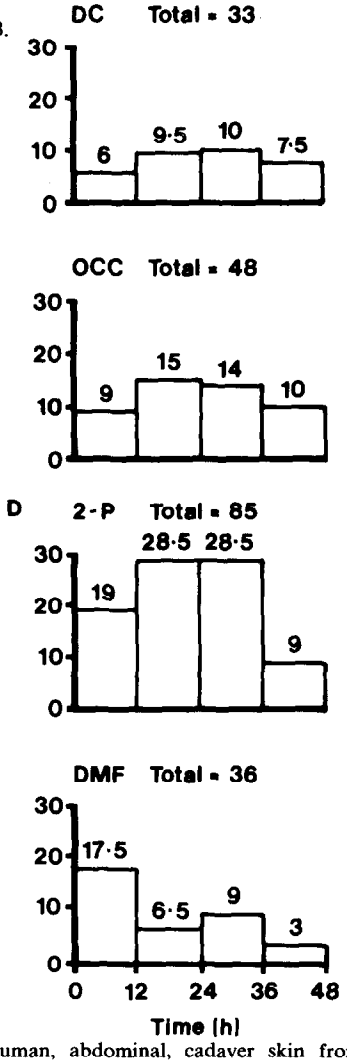

Fig. 2. Example plot for caffeine penetration through human, abdominal, cadaver skin from an acetone-deposited film under treatments: ●, dry control; ■, hydration (occluded); ○, 2-pyrrolidone; and □, dimethylformamide. (a) % dose penetrating 3.14 cm^2 (C) versus time. (b) Penetration rate (J, % dose·h^{-1}) versus time.

Fig. 3. Example histogram for caffeine penetration through human, abdominal, cadaver skin from an acetone-deposited film, under treatments: dry control (DC), hydration (occlusion-OCC), 2-pyrrolidone (2-P) and dimethylformamide (DMF), % Dose penetrating (D) versus time, Total = total % dose penetrated in 48 h.

corneum rapidly, exerting a more transient accelerant action than did 2-P.

For caffeine, hydration only increased J_{max} by an average factor of 2. However, this corresponds to an increase in the apparent diffusion coefficient of approximately 10-fold. This may be caused either by hydration-reducing interaction of the drugs with the membrane or by expanding the routes available for penetration. Behl et al. (1980) showed that the penetration of very polar alcohols was not increased by hydration of hairless mouse skin. If we assume that hairless mouse skin behaves similarly to human skin, we can conclude that as aspirin and caffeine penetrate more rapidly through hydrated skin then they probably pass, at least in part, through a lipid-rich route.

In previous steady-state diffusion experiments (Southwell and Barry, 1983) we equilibrated stratum corneum membranes on both sides with aqueous 2-P mixtures and found that this treatment did not alter appreciably the flux of caffeine relative to water conditioning. However, aqueous DMF increased the flux dramatically. We felt that this indicated either that the mode of action of the two solvents differed or that DMF damaged the membrane. Since in the present finite dose experiment 2-P and DMF act somewhat similarly (except for their time scales) it appears that small volume applications are not damaging.

Table 2 compares our control treatment data with similar results taken from the in vitro work of Franz (1975) and the in vivo work of Feldmann and Maibach (1970). For aspirin, our data agreed well with the results of these workers except that our J_{max} was twice that of Feldmann and Maibach. For caffeine, our measurement agreed more closely with the in vivo results than with the in vitro work.

Aspirin and caffeine readily penetrated normally hydrated skin; thus, the poten-

TABLE 1

FINITE DOSE, 'IN VIVO MIMIC' DIFFUSION OF ASPIRIN AND CAFFEINE IN HUMAN SKIN

Mean data reported for maximum flux, J_{max}; time of J_{max}, T_{max}; % dose penetrated in 48 h and apparent diffusion coefficient, D.

Treatment	n [a]	J_{max} (% dose·h^{-1})	T_{max} (h)	% Dose in 48 h	D [b] $\times 10^{12}$ (cm^2·s^{-1})
Caffeine					
Control	5	2.1	9.8	48	7.0
Hydration	5	4.9	8.4	80	80
2-P	5	7.4	10.4	90	–
DMF	5	9.1	3.8	61	–
Aspirin [c]					
Control	4	0.8	14	26	4.3
Hydration	2	1.3	28	35	46
2-P	4	5.8	16	59	–
DMF	2	2.2	5	73	–

[a] n = number of skin specimens.

[b] $D = h^2/6\ T_{max}$, where h = barrier thickness of 10 μm except for hydration when h = 30 μm.

[c] Aspirin uncorrected for hydrolysis.

TABLE 2

PENETRATION OF ASPIRIN AND CAFFEINE THROUGH HUMAN SKIN FROM SOLVENT DEPOSITED FILMS, (~ 4 $\mu g \cdot cm^{-2}$)

Data reported as maximum flux, J_{max}; time of J_{max}, T_{max}; and % dose penetrated, for in vivo data in 5 days, and for in vitro data in 48 h.

Data source	In vitro or in vivo	Aspirin [a]			Caffeine		
		J_{max} (% dose·h^{-1})	T_{max} (h)	% Dose	J_{max} (% dose·h^{-1})	T_{max} (h)	% Dose
Feldmann and Maibach	in vivo	0.44	12–24 (n = 3) [c]	22 (±3, n = 3) [b]	1.4	12–24 (n = 12)	48 (±21, n = 12) [b]
Franz	in vitro	0.85 (n = 1)	16–24	41 (17, 49, n = 12) [d]	0.75 (n = 1)	16–24	9 (5.5, 20, n = 17) [d]
This work	in vitro [b]	0.80 (±0.4, n = 4)	14 (±8, n = 4)	26 (±12, n = 4)	3.1 (±2.6, n = 6)	9.8 (±8.1, n = 5)	48 (±9.9, n = 5)

[a] Aspirin uncorrected for hydrolysis.

[b] ±S.D. given.

[c] n = sample number.

[d] 95% confidence interval given.

tial for demonstrating the action of penetration enhancers is limited. With molecules of much lower intrinsic penetrability, 2-P and DMF may produce more dramatic effects.

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