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Investigation of the *in vitro* interaction of various vehicles with hairless mouse skin

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Synopsis

The interaction of a series of donor solvents including water, propylene glycol, two alcohols, four hydrocarbons, and light mineral oil with hairless mouse skin was studied *in vitro* by following the dermal absorption of caffeine as a reference compound. Steady-state flux at saturation (J*) of caffeine was calculated for different donor solvents using solubility, donor concentration, and steady-state flux. The value of J* varied markedly with the donor solvent, indicating that caffeine permeation was significantly affected by vehicle/skin interactions. n-Propanol provided the highest value of J* among the various solvents. In the hydrocarbon series, the value of J* decreased exponentially as the chain length was increased. Propylene glycol yielded a lower value of J* compared to water. Light mineral oil provided the lowest value of flux at saturation, suggesting minimal interaction between this vehicle and mouse skin.

INTRODUCTION

In skin permeation studies, vehicles (solvents) are frequently employed to apply the test material(s) to the membrane surface. The physical and chemical properties of the vehicle play a major role in determining the rate of uptake and penetration of the medicament through the membrane (1). Vehicles can modify either the thermodynamic activity of the drug (2) or the barrier properties of the skin (3). Thus, a thorough understanding of the interaction of the solvent with the drug or the membrane is essential before its selection in any formulation for transdermal drug delivery as well as for dermal toxicological evaluation.

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Many therapeutic and cosmetic agents are nonpolar in nature with limited aqueous solubility. They readily dissolve in various organic solvents, but use of many of these vehicles is restricted in skin permeation experiments because of their membrane-damaging potential. The objective of the present study was to investigate the interaction of a series of donor solvents with full-thickness hairless mouse skin, using caffeine as a reference compound for skin permeation measurements. The octanol-water partition coefficient of this compound is 1 and it has adequate solubility in various polar and nonpolar solvents, thus making it a suitable candidate for this study.

MATERIALS AND METHODS

MATERIALS

Radiolabeled caffeine was utilized to facilitate skin permeation quantitation. [^{14}C] caffeine (specific activity, 55.7 mCi/mmol) was obtained from ICN Biomedicals, Costa Mesa, CA, and its radiochemical purity was determined by the manufacturer to be greater than 97%. The radioactivity of experimental samples was measured in a 1217 Rackbeta scintillation counter (LKB Instruments, Gaithersburg, MD) using Liquiscint® (Diagnostic Products Corp., Manville, NJ) as the scintillation fluid. Nonradiolabeled caffeine, chlorobutanol, light mineral oil, n-pentadecane, n-dodecane, n-nonane, n-heptane, propylene glycol, n-propanol, and isopropanol were obtained from either Sigma Chemical Co., St. Louis, MO, or Fisher Scientific, Springfield, NJ, and used as received.

METHODS

Preparation of skin samples. Female hairless mice (Skh 1, Charles River Labs, Wilmington, MA), 8–14 weeks old, were used as the source for all skin samples. Animals were sacrificed by cervical dislocation and skin was immediately excised. Subcutaneous fat and underlying tissues were carefully removed from the dermal surface. The skin samples were inspected visually for defects such as tears or holes and used within one half hour after animal sacrifice. Full-thickness skin, from both dorsal and ventral side, was used in initial experiments. The dorsal skin was selected for subsequent use for its ease of handling.

Preparation of donor solutions. Nine donor solvents were selected for investigating vehicle–skin interactions. These included: water, propylene glycol, isopropanol, n-propanol, n-heptane, n-nonane, n-dodecane, n-pentadecane, and light mineral oil. Radiolabeled caffeine was mixed with unlabeled compound in an alcoholic solution to obtain a final specific activity of 3.63 mCi/mmol. Different volumes of this solution were placed in glass vials, and following evaporation of alcohol, the residues were dissolved in selected volumes of various solvents to prepare donor solutions.

In vitro diffusion studies. A modified flow-through diffusion cell system, similar to that described by Bronaugh *et al.* (4) was utilized in skin permeation studies. The diffusional area in each cell was 0.64 cm^2. The receptor fluid continuously washed the dermal surface of the skin and was collected into vials placed in the fraction collector. Freshly degassed receptor solution was used in each experiment. The skin surface temperature was maintained at 32°C by adjusting the water bath temperature to 34.5°C. After an

initial equilibration period (one half hour), the donor was added to the skin sample mounted in the cell. The fraction collector was set to collect effluent for a desired time interval. Each cell was inspected at different time intervals for the presence of air bubbles under the skin surface. Any bubbles formed during the experiment were removed by tilting the cell. Depletion of the donor was avoided by total replacement with fresh donor solution periodically, as necessary, to maintain the concentration as essentially invariant. The experiment was continued until a sufficient number of samples were collected to allow estimation of the steady-state flux (J). At the end of the experiment, receptor samples, unpenetrated donor (plus cell washings), and skin samples were analyzed for caffeine content by liquid scintillation counting.

Data analyses. Raw radioactivity counts were converted to quantities of permeant using counting efficiencies and specific activities. Cumulative amounts of permeant penetrated per unit area were plotted against time and the linear portion of each plot was subjected to linear least square regression to determine steady-state flux and lag time (t_L) for that penetration experiment. The permeability coefficient (K_p) was calculated by dividing steady-state flux by the donor concentration of penetrant.

Caffeine solubility studies. The solubility of caffeine in various solvents was determined by adding an excess amount of nonradiolabeled caffeine to 10 ml of solvent in test tubes. The tubes were kept for one week in a water bath maintained at 32°C and shaken at regular intervals. The solutions were then filtered through 0.45 μm disposable filters and analyzed spectrophotometrically. Caffeine content was determined using calibration curves of caffeine constructed with the solvents.

Statistical analyses. All data were evaluated using analysis of variance (ANOVA). Differences between means were considered significant if p was less than 0.05.

RESULTS AND DISCUSSION

Experiments were carried out to determine the effects of receptor fluid flow rate on the permeation of caffeine through hairless mouse skin. The use of a low flow rate may give rise to a large stagnant layer and reduced dermal permeation. Two flow rates, 1.0 and 3.0 ml/hr, were selected, and permeation experiments were carried out using full-thickness dorsal skin and an aqueous caffeine solution as donor. As shown in Table I, steady-state caffeine fluxes obtained at both flow rates were statistically indistinguish-

Table I
Steady-State Fluxes of Caffeine Through Female Hairless Mouse Skin at Different Receptor Fluid Flow Rates From an Aqueous Donor Solution[a,b]

Animal	Flow rate (ml/hr)	Steady-state flux ($ng/cm^2/hr$)
1	1.0	3.83 (0.35)
	3.0	3.83 (0.12)
2	1.0	3.97 (0.25)
	3.0	4.10 (0.26)

[a] Mean of at least three determinations.
[b] Numbers in parentheses are standard deviations.

able. Thus, receptor fluid flow rates maintained at or greater than 1.0 ml/hr would ensure sink conditions during caffeine permeation experiments and a flow rate of 2 ml/hr was chosen for further studies.

Figure 1 compares the permeation profiles of caffeine through dorsal and ventral skin from an aqueous donor solution over a period of 24 hours. The steady state of permeation was reached at about 14 hours with both membranes. The average steady-state flux and lag time of caffeine permeation for different animals are shown in Table II. A two-way analysis of variance of the data indicated that there was no significant difference in steady-state flux values between dorsal and ventral skin. Also, variability among the animals used in the experiments was statistically insignificant. The dorsal skin was selected for further experiments because of its greater ease of handling. The permeability coefficient of caffeine permeation in hairless mice was 9.8×10^{-4} cm/hr; the published value in rats was 3.1×10^{-4} cm/hr (5).

Interaction of hairless mouse skin with different donor vehicles was studied using nine solvents. Table III shows the donor concentrations and average steady-state fluxes of caffeine from different donor media. The maximum steady-state flux of caffeine was obtained with n-heptane as the donor solvent. However, an increase in hydrocarbon

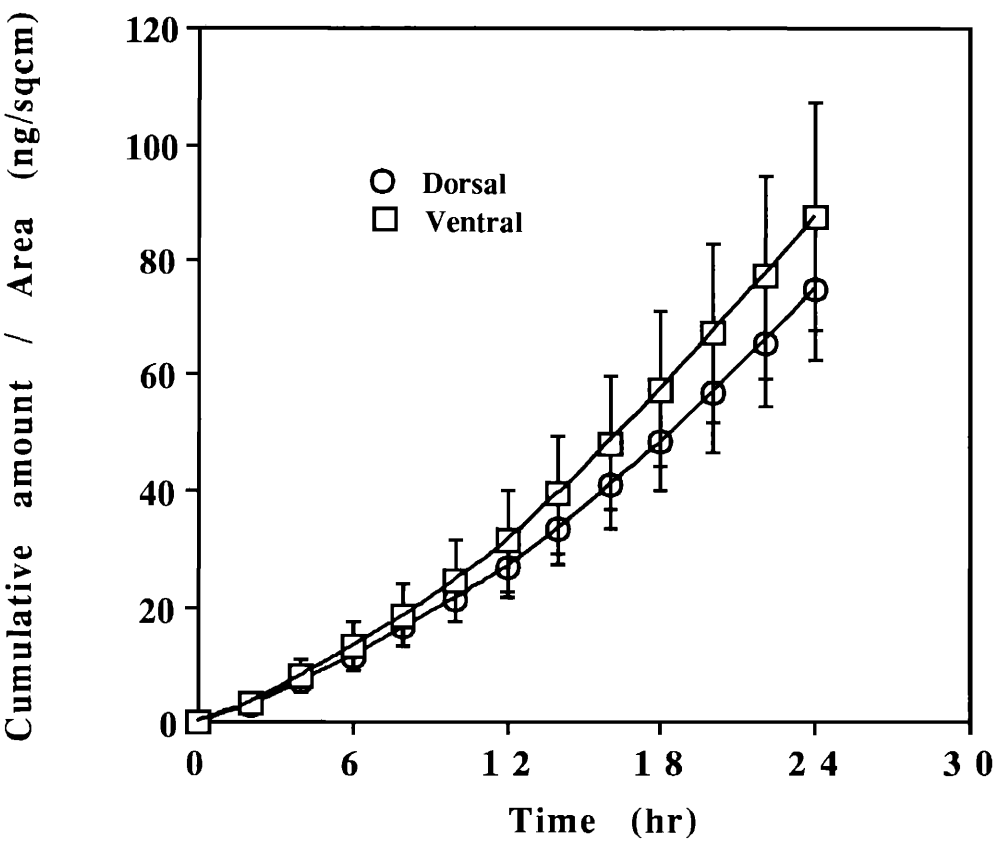

Figure 1. Permeation profiles of caffeine through female hairless mouse skin from an aqueous donor solution. Bars indicate S.D.

Table II
Steady-State Fluxes and Lag Times of Caffeine Through Female Hairless Mouse Skin From an Aqueous Donor Solution[a,b]

Animal	Skin type	Steady-state flux ($ng/cm^2/hr$)	Lag time (hr)
1	Dorsal	4.62 (0.40)	5.51 (0.41)
	Ventral	4.31 (0.49)	5.92 (1.18)
2	Dorsal	3.89 (0.61)	5.91 (0.42)
	Ventral	4.01 (0.69)	6.72 (0.51)
3	Dorsal	3.73 (0.52)	5.34 (1.13)
	Ventral	3.73 (0.59)	5.72 (1.31)

[a] Mean of at least three determinations.
[b] Numbers in parentheses are standard deviations.

chain length of the donor solvent caused a significant decrease in steady-state flux. n-Propanol provided a greater flux of caffeine than isopropanol. Propylene glycol yielded a slightly higher value of steady-state caffeine flux compared to water.

Solubilities of caffeine in different donor vehicles, determined at 32°C, also appear in Table III. Among the selected solvents, water provided the maximum solubility of caffeine. There was no significant difference in caffeine solubility between n-propanol and isopropanol. The solubility was relatively low in hydrocarbon vehicles.

Comparison of steady-state fluxes of caffeine from different donor media is not sufficient to assess vehicle–skin interactions, as the thermodynamic activity of the penetrant is not the same in various donor solutions. For a meaningful comparison, steady-state flux (J) values were converted to flux values at saturation (J*), using the following relationship:

$$J^* = (JS/C) \qquad \text{(Eq. 1)}$$

where C is the concentration of caffeine in the donor medium and S is the solubility. This relationship assumes that Fick's law holds over the entire concentration range.

Table III
Solubility (S), Donor Concentration (C), Steady-State Flux (J), and Flux at Saturation (J*) of Caffeine in Different Donor Solvents at 32 °C[a]

Solvent	$S \times 10^{-2}$ (μg/ml)	C (μg/ml)	J ($ng/cm^2/hr$)	J* ($\mu g/cm^2/hr$)
Water	268.7	4.07	3.81	24.92
Propylene glycol	117.8	5.00	5.99	12.83
n-Propanol	49.77	5.01	186.2	184.9
Isopropanol	49.71	5.00	53.32	52.98
n-Heptane	0.888	4.62	6528	125.5
n-Nonane	0.710	4.91	5080	73.43
n-Dodecane	0.684	4.51	2307	34.98
n-Pentadecane	0.699	5.01	1210	16.88
Light mineral oil	0.821	3.71	29.81	0.663

[a] Mean of at least three determinations.

The J* value for a donor solvent is an important parameter, as it indicates the degree of interaction between the solvent and the membrane. For saturated solutions containing excess permeant, the product of the partition coefficient and solubility in different donor solutions remains constant and does not change irrespective of vehicle (6). Therefore, differences in the values of J* reflect differences in diffusivity of caffeine caused by the interactions of various solvents with the membrane. Generally, the higher the value of J*, the more the interaction between the donor solvent and the membrane, independent of permeant solubility. The viscosities of various donor solutions were assumed not to significantly affect the steady-state permeation of caffeine, as diffusion through the mouse skin was the rate-determining step in the permeation process.

Table III shows J* values obtained for different solvents. The values of J* varied widely, indicating that vehicle–skin interactions contributed significantly to caffeine permeation. n-Propanol was found to have the highest value of J*, indicating the greatest interaction between this solvent and the mouse skin among the various solvents investigated. Isopropanol provided a much lower value of J* compared to n-propanol. The higher values of J* associated with these alcohols may be related to their ability to remove lipids from the stratum corneum (7,8).

In the hydrocarbon series (n-heptane, n-nonane, n-dodecane, and n-pentadecane), shorter chain length compounds had higher values of flux at saturation and, in fact, the value of J* decreased exponentially as the carbon chain length was increased, as shown in Figure 2. This indicated that shorter carbon chain length hydrocarbons exhibited greater interactions with the hairless mouse skin than the longer chain length compounds. Among the possible effects of short-chain hydrocarbons are increased fluidity of the intercellular lipid layer and extraction of lipids and/or other components. Light mineral oil (a mixture of liquid hydrocarbons from petroleum with an average hydrocarbon chain length of 30) provided the least interaction with the mouse skin, as indicated by the lowest value of J*. This solvent has been reported to be an inert, noninteracting, and nonpenetrating vehicle by other workers (2,9).

Propylene glycol yielded a lower value of J* compared to water. There are conflicting reports regarding the effect of propylene glycol on the skin penetration of compounds (10–17). Incorporation of propylene glycol in the vehicle has been found to enhance the percutaneous absorption of various chemicals including fluocinolone acetonide (10), betamethasone valerate (11), diflorasone diacetate (12), trifluorothymidine (13), and estradiol (14). On the other hand, dermal absorption of chloramphenicol (15), butylparaben (16), and theophylline (17) is decreased when propylene glycol is added to the vehicle. Propylene glycol is a hygroscopic solvent, and it may cause dehydration of the skin; this effect has been suggested to be responsible for the decreased transdermal permeation of chemical from this vehicle (18). This phenomenon may be operative in the present study. While J* values for propylene glycol are equal to or less than that for water when compared under infinite dose conditions, as seen in the present study, similar results may not be obtained under open conditions that permit rapid evaporation of volatile solvents and formation of thin films on the skin surface (19).

In conclusion, donor solvents used in skin permeation studies interact with the membrane, thereby altering its barrier properties. The extent of this interaction varies widely among solvents, as seen in the present study. Selection of the appropriate donor medium is essential when investigating skin penetration characteristics of a compound.

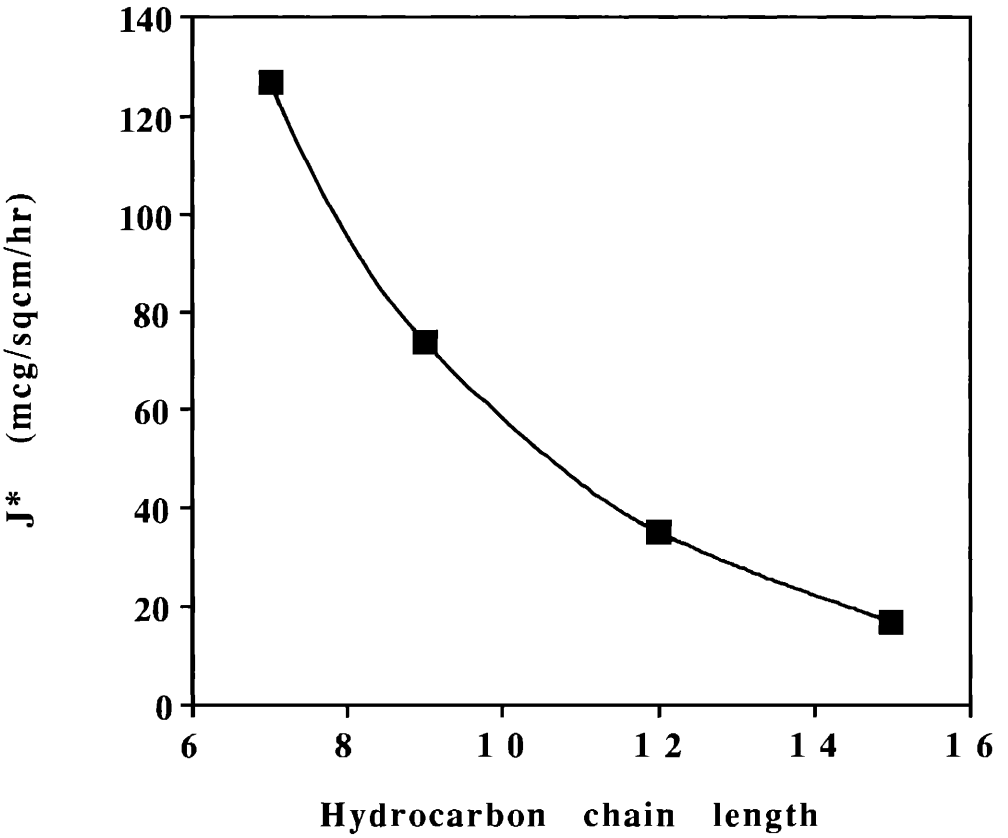

Figure 2. Effect of chain length of the hydrocarbons on the calculated steady-state flux at saturation (J*) of caffeine through female dorsal hairless mouse skin.

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