ELSEVIER

International Journal of Pharmaceutics 156 (1997) 17–26

international journal of pharmaceutics

Alcohol flux and effect on the delivery of theophylline from propylene glycol

K.B. Sloan *, H.E. Taylor, J.C. Hamilton

Department of Medicinal Chemistry. P.O. Box 100485, University of Florida, Gainesville, Florida 32610, USA

Received 14 February 1997; received in revised form 30 April 1997; accepted 29 May 1997

Abstract

The diffusion of alcohols and the back-diffusion of water through hairless mouse skin after the application of alcohols have been determined using ^{1}H NMR spectroscopy to quantitate the composition of the donor phases during diffusion cell experiments. After the alcohols were removed, a second application of a standard solute/vehicle (theophylline/propylene glycol-PG) was made. The diffusion of PG and the back-diffusion of water were determined using ^{1}H NMR spectroscopy to quantitate the composition of the donor phases while the diffusion of theophylline was determined using UV spectroscopy to quantitate theophylline in the receptor phases. The flux values for theophylline, PG and water were used as a measure of irreversible damage done to the skins by the initial application. The flux of the alcohols and the back-diffusion of water in the first application period decreased with increasing chain length of the alcohol. On the other hand, the flux of theophylline, PG and water in the second application increased with increasing chain length of the alcohol that had been applied initially. The results from the second application appear to be due to greater irreversible damage caused by the longer chain alcohols. There is a clear difference between the extent of damage caused by the C_1 to C_3 alcohols and that caused by the C_4 to C_8 alcohols. © 1997 Elsevier Science B.V.

Keywords: Alcohols; ^{1}H NMR spectroscopy; Diffusion cell; Transdermal delivery; Theophylline; Hairless mouse skin

1. Introduction

Simple aliphatic alcohols are among the most thoroughly studied vehicles used to deliver permeants into and through skin. However, there are inconsistencies in the results reported from one group to another. For example, Scheuplein and Blank (1973) reported that the flux of alcohols through human skin (either epidermis or dermis) decreased as the chain length increased from C_1 (methanol) to C_{10} (decanol). A different trend was obtained when the fluxes of the alcohols from aqueous solutions were measured. For the epidermis, the fluxes of the shorter-chain alcohols (C_1–

* Corresponding author.

PII S0378-5173(97)00179-8

C_5), which were all tested at 0.1 M and were soluble to the extent of at least 0.1 M, increased as the chain length increased and saturation was approached. This was expected since maximum flux should be observed from the saturated solution of a solute in a vehicle (Ostrenga et al., 1971; Cooper, 1984). The longer-chain alcohols (C_6–C_{10}) were tested as saturated solutions (equal chemical potentials) and the fluxes of the alcohols through the epidermis decreased as the chain length increased. On the other hand, alcohol fluxes from the same aqueous solutions through the dermis decreased in a regular manner as the chain length of the alcohol increased starting with the C_1 alcohol.

Portions of these results by Scheuplein and Blank (1973) have been reproduced by some authors while others have reported somewhat different results. Goldberg-Cettina et al. (1995) have examined the effect of short-chain alcohols and their mixtures with isopropyl myristate on the delivery of estradiol through human epidermis and the disposition of the alcohols during the experiments. For the neat alcohol vehicles that were examined (ethanol, 1-propanol and 1-octanol), alcohol flux was greatest for ethanol, was about 10-fold less for 1-propanol and was not detectable for 1-octanol. These results are in general agreement with those of Scheuplein and Blank (1973).

On the other hand, Kai et al. (1990) have examined the C_2–C_{12} 1-alcohols when applied to hairless mouse skins and their effect on the subsequent delivery of nicotinamide from water through the same skins. They found that the flux of alcohol through skin increased in going from the C_2 to C_4 alcohol by 3-fold and then decreased in going from the C_4 to C_8 alcohol by 25-fold. Although the amount of alcohol found in the skin increased by 7-fold in a somewhat regular manner in going from the C_2 to C_{10} alcohol, the trend in the total amount of alcohol that permeated into and through the skin was the same as the trend in the flux through the skin. This is because the amount of alcohol that permeated through the skin was up to 10-fold greater than that permeated into the skin for the C_2–C_5 alcohols. The trend observed for the C_2–C_4 alcohols is not in agreement with those of Scheuplein and Blank (1973) or Goldberg-Cettina et al. (1995).

The delivery of the polar permeant nicotinamide from water through hairless mouse skins after they had been pretreated with the C_2–C_{12} alcohols exhibited a trend similar to that exhibited by the alcohols: the delivery of nicotinamide increased 13-fold in going from pretreatment with the C_2 to pretreatment with the C_6 alcohol, then decreased 9-fold in going from the C_6 to C_{12} alcohol (Kai et al., 1990). Similarly, for the lipophilic permeant levonorgestrel, Friend et al. (1988) reported a 10-fold increase in the delivery of levonorgestrel in going from the C_2 to C_4 alcohol then a decrease of 35-fold in going to delivery from the C_8 alcohol. These two results suggest that the effect of alcohols on the delivery of a permeant may give the same trend regardless of when the alcohols are applied and regardless of the polarity of the permeant.

The following experiments were designed to determine the disposition of alcohols applied to hairless mouse skin in diffusion cell experiments using ^{1}H NMR spectroscopy to quantitate alcohol and H_2O in the donor phases. The results will be compared with those obtained by Scheuplein and Blank (1973) and Kai et al. (1990) where the concentration of radiolabeled alcohol in the receptor phase was measured. Theophylline (Th) in propylene glycol (PG) was chosen as the model solute/vehicle for the second application experiments to extend previous results obtained in this laboratory which used Th/PG to compare the effect of vehicles on the delivery of theophylline from vehicles containing different functional groups (Sloan et al., 1986). In addition, the use of a nondamaging vehicle (PG) (Sloan et al., 1986) other than water makes it possible to use ^{1}H NMR spectroscopy to determine the back-diffusion of water into the donor phase in the second application studies. These results will be compared with those of Kai et al. (1990).

2. Experimental

2.1. Materials

Ultraviolet (UV) spectra were run on a Cary 210 or a Shimadzu UV-265 spectrophotometer. ^{1}H NMR spectra were obtained at 90 HMz on a Varian EM-390 spectrometer. 1-Octanol and propylene glycol (PG) were $>99\%$ pure, and the 1-hexanol and 1-heptanol were $>98\%$ pure from Aldrich Chemical Co. 1-Butanol and 1-pentanol were reagent grade solvents from Mallinckrodt. 1-Propanol was analyzed reagent grade from J.T. Baker Chemical Co. Absolute ethanol was obtained from Aaper Alcohol and Chemical Co., Shelbyville, Kentucky. Methanol was certified ACS grade from Fisher Scientific. Analysis of the ^{1}H NMR spectra of the alcohols showed that they did not contain any water. The diffusion cells were glass Franz type from Crown Glass of Somerville, New Jersey (surface area 4.9 cm^2, 20 ml receptor phase volume). The diffusion cells were maintained at 32°C with a Fisher circulating waterbath Model 25. The female hairless mice (25–28 g, 12–16 weeks old, SKH-hr-1) were from Charles River. The anhydrous theophylline (Th) and DMSO-d_6 in 10 g glass ampules were purchased from Aldrich Chemical Co. The DMSO-d_6 ampules were opened immediately before spectra were run and stored in a vacuum desiccator between spectra.

2.2. Diffusion cell experiments

The diffusion cell experiments were run according to previously described procedures (Sloan et al., 1986). In addition, the donor phases were also periodically removed, weighed and analyzed by ^{1}H NMR spectroscopy.

Briefly, female hairless mice were sacrificed by cervical dislocation. Their skins were removed by blunt dissection and placed epidermal side up in glass Franz diffusion cells with the dermal side in contact with pH 7.1 phosphate buffer (0.05 M, $I = 0.11$ M, 32°C) containing 0.11% formaldehyde (2.7 ml of 36% aqueous formaldehyde/liter) to prevent microbial growth and to insure the integrity of the mouse skins during the course of the experimental: Th flux from PG applied 4 h after sacrifice was $6.1 \pm 0.6 \times 10^{-3}$ μmol cm^{-2} h^{-1}; 24 h after sacrifice was $8.3 \pm 1.9 \times 10^{-3}$ μmol cm^{-2} h^{-1}; 48 h after sacrifice the value was $9.4 \pm 1.2 \times 10^{-3}$ μmol cm^{-2} h^{-1}; 120 h after sacrifice was $10.0 \pm 1.2 \times 10^{-3}$ μmol cm^{-2} h^{-1} (Sloan et al., 1991). The skins were kept in contact with the buffer for at least 48 h to condition the skins and to allow UV absorbing materials to leach from the skins; the receptor phases were changed at least three times during this time to facilitate the leaching process.

2.2.1. First application

During the first application period of 48 h, 1.0 ml of each neat alcohol was applied to the epidermal surface of three hairless mouse skins using a calibrated Eppendorf digital pipette. The weight of alcohol delivered to each skin was determined as the average of repeated ($n = 4$) weighings of 1.0 ml samples of each alcohol. The donor chamber of each diffusion cell was then sealed with parafilm and kept sealed during each application period except when the donor phase and the receptor phase were changed. Care was taken to ensure that any condensate on the bottom of the parafilm was returned to the donor phase each time the parafilm was removed. The condensate from the application of the C_4–C_8 alcohols appeared to be primarily water since the second phase in the donor phase was not observed until after the condensate had been returned to the donor phase. Control experiments showed that parafilm was effective in retaining 97–99% of 1.0 ml samples of C_1–C_4 alcohols in 50 ml beakers ($n = 3$) over a 24 h period and that no water was absorbed. These experiments confirmed that the alcohols did not evaporate and that water was not absorbed from the atmosphere by the alcohols.

The donor phases from each diffusion cell were removed at 12, 24, 36 and 48 h using a disposable pipet and bulb suction, weighed and analyzed by ^{1}H NMR spectroscopy. The donor phase was immediately replaced with fresh neat alcohol after 12, 24 and 36 h. Control experiments showed that between 99 and 100% of the applied alcohols were removed from hairless mouse skins using a disposable pipet and bulb suction ($n = 4$), except for

PG where 96.6% was removed. If the donor phases contained two phases, they were allowed to settle for 48 h in tightly sealed, weighed glass test tubes before the phases were separated using a pipet and weighed by difference.

Small portions (approximately 100 μl) of these separated donor phases were then dissolved in DMSO-d_6 for analyses by ^{1}H NMR spectroscopy. The three individual water phases obtained at each sampling interval for the C_4–C_7 alcohols were each too small to allow accurate measurements of alcohol in each sample by ^{1}H NMR spectroscopy so the water phases were combined and then analyzed. The integration due to the H_2O absorption in each ^{1}H NMR spectrum of the alcohol phase was determined from the total integration at approximately δ3.0–3.5 minus (a) the integration of the residual H_2O in the DMSO-d_6 determined immediately before the donor phase sample was added (this integration amounted to less than 1% of total integration), and (b) the integration for CH_2OH from the alcohol. The integration for CH_2OH from the alcohol was calculated from $2 \times CH_2OH$ for the C_5–C_7 alcohols or from $2 \times CH_3/3$ for the C_1 to C_4 alcohols. For PG the integration in part (b) was for CH_2OH and CHOH and was calculated from $3 \times CH_3/3$; the position of the H_2O absorption also increased from approximately δ3.5 to 4.5 as the amount of H_2O in the donor phase increased. The apparent ratio (R) of the integration due to the $H_2O/2$ absorption to the integration due to the CH_2OH (for the C_5–C_8 alcohols) or to the $CH_3/3$ absorption (for the C_1–C_4 alcohols) gave the molar ratio of H_2O to alcohol in each separate phase. In control experiments, mixtures of alcohol and H_2O in known ratios (R_1) similar to those observed in the ^{1}H NMR spectra of the donor phases were analyzed by ^{1}H NMR spectroscopy to give measured ratios (R_2) for the known compositions. The correction factor R_1/R_2 was then multiplied by R to give a corrected ratio (CR). From CR, the weight of alcohol and water in the phase were determined by solving the following equation for X (moles of alcohol) where MW is the molecular weight of the alcohol:

$$\text{MW(alcohol)}X + \text{CR18}X = \text{weight of phase}$$

After the donor phases from the first application were removed, the donor surfaces were quickly washed with 3×5 ml portions of methanol. After the methanol wash, the skins were kept in contact with fresh receptor phase for 23–24 h to allow any alcohol that had been absorbed to leach out of the skins (Siver and Sloan, 1988).

2.2.2. Second application

After the 23–24 h leaching period, the receptor phases were replaced and 0.5 ml aliquots of a model solute/vehicle donor phase suspension (theophylline-Th/propylene glycol-PG: 67 mg ml^{-1}) were applied to each skin. The weight of theophylline in the suspension was determined by UV spectroscopy ($\epsilon = 1.02 \times 10^4$ l mol^{-1} at 270 nm) (Sloan et al., 1986). The weight of PG in the 0.5 ml aliquot (0.51 g) was calculated from the difference in the weight of the suspension and the weight of theophylline in the suspension assuming a uniform dispersion ($n = 4$). The suspensions were kept well-stirred and aliquots for the donor phases and weighings were removed from the middle of the well-stirred suspensions during sampling. This second application period was for 4 h. Receptor phase samples were typically removed at 1, 2, 3 and 4 h: theophylline was quantitated by UV spectroscopy as above. Each time a sample was removed, the entire receptor phase was replaced. At 4 h the donor phases were removed and allowed to settle for 48 h. The supernatants were separated from the residue using a pipet, weighed by difference and analyzed by ^{1}H NMR spectroscopy as above. The corrected ratio (CR) of the integration of the $H_2O/2$ absorption (minus residual H_2O in the DMSO-d_6 and CHOH and CH_2OH absorptions as above) to the $CH_3/3$ absorption gave the molar ratio of H_2O to PG in the donor phase. From CR, the weight of PG and H_2O in the phase could be determined by solving the following equation for X (moles of PG):

$$76X + \text{CR18}X = \text{weight of phase}$$

2.2.3. Flux calculations

The flux (J) of theophylline was determined by plotting the cumulative mg of theophylline mea-

Table 1
Estimated disposition of alcohol and water during alcohol application ($n = 12$): first application

Alcohol	Alcohol applied[a]	Alcohol diffused in 12 h[a]	Water absorbed in 12 h[b]	Alcohol flux[b]	Water back-flux[b]
CH_3OH	0.79	0.47 (0.03)	0.32 (0.04)	253 (14)	306 (36)
C_2H_5OH	0.78	0.37 (0.04)	0.32 (0.08)	137 (14)	300 (73)
C_3H_7OH	0.81	0.44 (0.06)	0.28 (0.05)	124 (16)	263 (42)
C_4H_9OH	0.80	0.39 (0.02)	0.18 (0.02)	89 (5)	167 (19)
$C_5H_{11}OH$	0.81	0.21 (0.04)	0.086 (0.01)	40 (7)	81 (13)
$C_6H_{13}OH$	0.81	0.068 (0.044)	0.104 (0.02)	11 (7)	98 (22)
$C_7H_{15}OH$	0.81	0.030 (0.014)	0.071 (0.031)	4 (2.2)	66 (30)
$C_8H_{17}OH$	0.81	N.D.[c]	N.D.	N.D.	N.D.

[a] Units of g ($\pm$ S.D.).
[b] Units of μmol cm^{-2} h^{-1} ($\pm$ S.D.) calculated from (average of amounts of alcohol diffused from or water absorbed into the donor phase from four consecutive applications of alcohol to each of three cells)/[(4.9 cm^2)(12 h)(molecular weight of alcohol or water)].
[c] N.D., not determined.

sured in the receptor phase against time and dividing the slopes of the steady-state portions of those plots by the surface area of the diffusion cells. The flux of alcohol or PG was estimated by dividing the μmol of alcohol or PG lost from the donor phase by time (12 or 4 h, respectively) and surface area of the cell (4.9 cm^2). The back-diffusion flux of water was estimated by dividing the μmol of water found in the donor phase by time (12 or 4 h, respectively) and surface area of the cell (4.9 cm^2).

3. Results and discussion

3.1. Application of alcohol

The donor phases from the application of the C_1–C_3 alcohols were homogeneous while the donor phases from the application of the C_4–C_8 alcohols were biphasic at the end of each application period. The amounts of alcohol and of water in the homogeneous donor phases or in the lipid portion of the biphasic donor phases were calculated from the intensities of the CH_3/3 (or CH_2OH) and H_2O/2 absorptions, respectively, in their ^{1}H NMR spectra. The amount of alcohol that had permeated (diffused into and through) the skin was the difference between the amount of alcohol applied and the amount measured in the donor phase at the end of each 12 h period. For the homogeneous donor phases, the amount of water that had undergone back-diffusion into the donor phase was the amount measured by ^{1}H NMR spectroscopy, while the amount of water that had undergone back-diffusion into the biphasic donor phases was the sum of the amount of water in the lipid portion of the donor phase and the amount in the separate water phase. The separate water phases were not observed for the C_4–C_8 alcohols until after the condensate on the parafilm cover had been returned to the donor phases. Thus, the water phases did not decrease the surface area of the skins available for partitioning of the alcohols into the skin.

In the case of the application of 1-butanol, the separate water phase was also analyzed by ^{1}H NMR spectroscopy. The amount of 1-butanol in the separate water phases only added 2% to the calculated amount of 1-butanol in the donor phase. Since the solubilities of the longer chain alcohols in water were lower (Smith et al., 1975) and smaller separate water phases were isolated in the experiments where they were applied, the contribution of alcohol in the separate water phases to the total donor phase concentration of alcohol was negligible. Similarly, since the solubilities of the longer chain alcohols in water was less than 2%, subtracting the amount of alcohol in the water phase had a negligible effect on the calculation of the back-diffusion of water.

Table 1 gives the fluxes of alcohol into and through three hairless mouse skins from each of four consecutive 12 h application periods ($n = 12$).

Table 2
Comparison of alcohol fluxes from other studies

Alcohol	Fluxes (μmol cm^{-2} h^{-1})		
	Scheuplein and blank	Goldberg-Cettina et al. (1995)	Kai et al. (1990)
CH_3OH	259	—	—
C_2H_5OH	12.3	17.0	74
C_3H_9OH	2.1	1.3	72
C_4H_9OH	0.65	—	139
$C_5H_{11}OH$	0.47	—	107
$C_6H_{13}OH$	0.43	—	39
$C_7H_{15}OH$	0.18	—	20
$C_8H_{17}OH$	0.063	N.D.[b]	16

[a] Estimated from Fig. 4 and Fig. 5 using [(total % dose absorbed into and through skin/100) (10^6)(0.5 ml)(density, g/ml)]/[(molecular weight)(area = 0.785 cm^2)(time = 6 h)] = flux in μmol cm^{-2} h^{-1} (Kai et al., 1990).
[b] N.D., Not detectable.

Although there was a trend to slightly higher flux values with time from the data at each 12 h period for some alcohols, there was no consistent trend for the whole series. Examples of estimated flux values at 12, 24, 36 and 48 h are as follows: $C_1 = 238$, 253, 253 and 267 μmol cm^{-2} h^{-1}, respectively; $C_2 = 120$, 137, 139 and 152 μmol cm^{-2} h^{-1}, respectively; $C_3 = 105$, 116, 140 and 134 μmol cm^{-2} h^{-1}, respectively; $C_4 = 89$, 86, 91 and 88 μmol cm^{-2} h^{-1}, respectively, $C_5 = 50$, 38, 35 and 37 μmol cm^{-2} h^{-1}, respectively. Each of these flux values is the average of three skins for that time period and the flux values given in Table 1 are the average of all 12 individual values. The reason that there was no significant lag time for development of flux is that the fluxes were calculated from disappearance of alcohol from the donor phase rather than appearance in the receptor phase. Thus, any hold-up in the skin would not have been detected.

A flux of C_8 alcohol or a concomitant back-flux of water could not be determined because values of alcohol diffused and water absorbed were so small they could not be reproducibly determined using ^{1}H NMR spectroscopy. This result is similar to that reported by Goldberg-Cettina et al. (1995) where no C_8 alcohol could be detected in the receptor phase. The trend in these calculated flux values shows the same trend in alcohol diffusion as that observed by Scheuplein and Blank (1973), i.e. the flux of the alcohol decreased as the chain length of the alcohol increased (Table 2). However, the flux through hairless mouse skin did not decrease (63-fold) as much as that through human skin (1440-fold) over the series of alcohols studied (Table 1 versus Table 2). Thus, the flux values for the C_3–C_7 alcohols were 130–25-fold greater through hairless mouse skin, and only the flux value for the C_1 alcohol was similar. This suggests that hairless mouse skin may be similar to human skin in its resistance to permeation by more polar solutes (CH_3OH), but is much more permeable to more lipoidal solutes.

The calculated values for flux in Table 1 include the amounts of alcohol that permeated into and through the skin because they are based on the amounts lost from the donor phase. In order to compare the flux values calculated here with those reported by Kai et al. (1990), their percent of dose values for alcohol uptake into and absorption across hairless mouse skin have been combined and converted into flux values reflecting total loss of alcohol from the donor phase (Table 2). The result is that for the C_4–C_7 alcohols, the trend in flux values is identical to the flux values in Table 1, except that the absolute values for flux reported by Kai et al. (1990) become progressively larger than the values reported in Table 1 as the chain length increased. However, the most significant difference between the two studies lies in the trend for the flux values for the C_2 and C_3 alcohols. The trend to decreased fluxes for shorter-chain alco-

hols reported by Kai et al. (1990) is opposite to the trend reported by Scheuplein and Blank (1973) and by Goldberg-Cettina et al. (1995) (Table 2) using human skin, and here (Table 1) using hairless mouse skin. The reason for this difference in the trend reported by Kai et al. (1990) is not obvious in light of the qualitative agreement among all the reports on the trend for the longer-chain alcohols.

The difference between the trend in these results and those of Kai et al. (1990) was not due to the fact that they applied the alcohols immediately after the mice were sacrificed and the mouse skins had been placed in the diffusion cells in contact with the receptor phase. In a separate experiment, 1.0 ml of each of the C_1–C_4 alcohols ($n = 2$) were applied immediately and the donor phases were removed after 6 h to reproduce their general procedure. Analysis of the donor phases from that separate experiment by ^{1}H NMR spectroscopy showed the same trend as the results from the remainder of this study: CH_3OH, $J_{ROH} = 292$ μmol cm^{-2} h^{-1} (11 μmol cm^{-2} h^{-1} = range); C_2H_5OH, $J_{ROH} = 90$ μmol cm^{-2} h^{-1} (2 μmol cm^{-2} h^{-1} = range); C_3H_7OH, $J_{ROH} = 90$ μmol cm^{-2} h^{-1} (11 μmol cm^{-2} h^{-1} = range); C_4H_9OH, $J_{ROH} = 48$ μmol cm^{-2} h^{-1} (6 μmol cm^{-2} h^{-1} = range). The flux values for the C_2 and C_3 alcohols are very close to those extracted (Table 2) from the data reported by Kai et al. (1990). The fact that the alcohol flux values for the C_3 and C_4 alcohols are somewhat lower in the separate experiment than the values in Table 1 may be expected because of the known effect of hydration of skin membranes on the permeation of more hydrophobic alcohols from dilute aqueous solutions (Behl et al., 1980). However, on that basis the flux of the C_2 alcohol should not have been affected (Behl et al., 1980) but it has. The fact that the flux of the C_2 alcohol is lower in the separate experiment may be due to the fact that the pure alcohol instead of a dilute aqueous solution was used in all of these experiments.

Table 1 also gives values for the flux of water into the donor phase across three hairless mouse skins (back-diffusion) from each of the four consecutive 12 h alcohol application periods. The water flux values from the separate experiment (see above) are very similar to those obtained from application of the C_1 and C_4 alcohols (for C_1, $J_{H2O} = 366$ μmol cm^{-2} h^{-1}, 17 μmol cm^{-2} h^{-1} = range; for C_4, $J_{H20} = 153$ μmol cm^{-2} h^{-1}, 1 μmol cm^{-2} h^{-1} = range), but much lower than those obtained from application of the C_2 and C_3 alcohols ($J_{H2O} = 133$ and 110 μmol cm^{-2} h^{-1}, respectively; 11 and 23 μmol cm^{-2} h^{-1} = range, respectively). The calculated flux values for back-diffusion of water for all the experiments suggest a qualitative difference between the effect of the application of the C_1–C_3 alcohols and the C_5–C_7 alcohols on back-diffusion with the C_4 alcohol having an intermediate effect. In this regard, it should be noted that the C_1–C_3 alcohols are miscible with water, thus providing more driving force for back-diffusion while the remaining alcohols are not.

The back-diffusion of water into the donor phase observed here is a phenomena that has been reported previously, e.g. Kurihara-Bergstrom et al. (1986). In fact, the back-diffusion is so extensive here that water becomes 50 to 43% of the donor phase at the end of each 12 h application period from the application of the C_1–C_3 alcohols. The extent of this back-diffusion of water does not seem to have been previously appreciated (Zatz and Dalvi, 1983).

3.2. Application of theophylline/propylene glycol after application of alcohol

In order to determine the effect of alcohol on the permeation of a subsequently applied solute, a suspension of theophylline in propylene glycol (PG) was applied to each skin 24 h after the fourth of the consecutive alcohol applications had been removed. This allowed time for the alcohol that was in the skin to leach out of the skin. Previous experiments had shown that this time interval was sufficient to allow $>90\%$ of polar permeants such as 6-mercaptopurine to leach from skin under similar conditions (Siver and Sloan, 1988). However, it was not established whether a longer leaching period was necessary to remove more lipophilic permeants. A suspension of theophylline in PG was applied so that a saturated solution of theophylline would be main-

tained in contact with the skin during the course of the experiments. Control experiments using suspensions containing twice the total concentration of theophylline in PG showed no significant differences in fluxes from those suspensions. Thus, dissolution of theophylline was not rate limiting (Higuchi, 1960).

The effect of the alcohols on the flux of subsequently applied theophylline/PG (Table 3) is similar to, but more pronounced than, the effect observed by Kai et al. (1990) on the flux of subsequently applied nicotinamide/water. In each case, the flux of solute was low after pretreatment with the short-chain (C_1–C_3) alcohols then increased with the medium-chain (C_4–C_8) alcohols. In the present work there was a sharp break in the effect on the flux of theophylline between the C_1–C_3 alcohols and the medium-chain alcohols which amounted to an increase of 50–600-fold in the flux of theophylline after pretreatment with the medium-chain alcohols. This effect amounted to only a 10–15-fold increase in the flux of nicotinamide from water after 6 h of pretreatment with the alcohols. The difference in the intensity of the effect may be due to the 8-fold greater total length of time that the alcohols were applied in the present work. This conclusion is supported by the fact that Kai et al. (1990) saw an increase in the effect of alcohol pretreatment on nicotinamide flux when the time of application was increased from 3 to 6 h. However, it should also be noted that in this work there was a 24 h leaching period between pretreatment and application of theophylline/PG, while Kai et al. (1990) applied nicotinamide/water immediately after pretreatment. The 24 h leaching period may have given sufficient time for the more polar C_1–C_3 alcohols, but not the more lipoidal C_4–C_8 alcohols, to leach from the skin in the present study and accentuate the difference between the short- and medium-chain alcohols.

Table 3
Estimated disposition of propylene glycol (PG) and H_2O during the application of theophylline (Th)/PG and transdermal delivery of Th ($n = 3$): second application

Alcohol	Th flux[a]	H_2O back-flux[a]	PG flux[a]
CH_3OH	0.006 (0.002)	225 (48)	112 (41)
C_2H_5OH	0.020 (0.001)	402 (12)	56 (27)
C_3H_7OH	0.071 (0.008)	845 (42)	36 (10)
C_4H_9OH	3.30 (1.7)	4200 (1400)	158 (20)
$C_5H_{11}OH$	5.58 (0.47)	4893 (304)	242 (28)
$C_6H_{13}OH$	4.02 (0.53)	4227 (272)	234 (11)
$C_7H_{15}OH$	4.48 (2.02)	4403 (1369)	228 (40)
$C_8H_{17}OH$	6.07 (1.43)	1666 (276)	275 (24)
Control	0.010 (0.0012)		

[a] Units of μmol cm^{-2} h^{-1}.

In addition to the flux of theophylline, the disposition of PG and water in the donor phase was also measured. The trends in the values for the flux of PG and the back-diffusion of water are the inverse of the trends observed for the flux of the alcohols and back-diffusion of water in the pretreatment part (Section 3.1) of the experiments. Instead, the trend in PG flux and back-diffusion of water follows the trend in flux of theophylline. There was a definite increase in the flux of PG and back-diffusion of water for the diffusion cells to which the medium-chain length (C_4–C_8) alcohols had been applied. In fact, a substantial amount of the PG had diffused from the donor phase in only 4 h after pretreatment with the medium-chain alcohols. Thus, the donor phases after the application of theophylline in PG varied from 16% PG after pretreatment with the C_4 alcohol to 8% or less PG after pretreatment with the remaining medium-chain alcohols. On the other hand, after pretreatment with the C_1, C_2 and C_3 alcohols, 81, 75 and 61%, respectively, of the donor phases were PG. Thus, the greater fluxes of theophylline and PG (a fairly polar solute and solvent) after pretreatment of the skins with the medium-chain alcohols supports the conclusion that application of the medium-chain C_4–C_8 alcohols makes skin less resistent to permeation by polar (theophylline and PG) molecules (see below).

No perceptible change in donor phase volume in the second application part of the experiments

was observed for the diffusion cells which had been pretreated with the short-chain alcohols. On the other hand, at least a doubling of donor phase volume was observed for the diffusion cells which had been pretreated with the medium-chain alcohols. All of these results suggest that there is a significant difference between the apparent reversible effect of the short-chain C_1–C_3 alcohols and the apparent irreversible effect of the medium-chain C_4–C_8 alcohols on the permeability of hairless mouse skin.

The fact that the fluxes of theophylline and of nicotinamide, both polar permeants, were increased after pretreatment of hairless mouse skin with the medium-chain alcohols suggests that the medium-chain alcohols compromise the ability of skin to resist permeation by polar molecules. Thus, although it was not possible to observe any lipid components of the membrane in the donor phases from treatment with the medium-chain alcohols by ^1H NMR spectroscopy because of interference from the CH_3 and CH_2 absorptions due to the alcohols, this work supports the conclusion of Kai et al. (1990) that extraction of skin lipids is the mechanism by which alcohols promote transdermal permeation. If the extraction mechanism is correct, as skin lipids are extracted the lipid component of the resistance of the skin to permeation is decreased making it easier for polar molecules to permeate. Moreover, the results of the present investigation suggest that this mechanism operates primarily for the medium-(compared to the short) chain length alcohols in which skin lipids should be more soluble.

4. Conclusions

The trend in the fluxes of pure alcohols through hairless mouse skins is similar to that through human skin reported by Scheuplein and Blank (1973). However, the flux through human skin by the medium-chain, more lipophilic alcohols decreased much more rapidly than their flux through hairless mouse skin. This suggests that the resistance of human skin is more similar to the resistance of hairless mouse skin to permeation by polar molecules than it is to permeation by more lipophilic molecules.

Although the short-chain alcohols (C_1–C_3) permeated hairless mouse skin more readily than the medium-chain alcohols (C_4–C_7), the short-chain alcohols caused much less irreversible damage to the skins as determined by second application studies using theophylline/PG as a standard solute/solvent. Not only was there a significant increase in flux values for theophylline, but there was also a significant increase in flux values for PG and back-diffusion values for H_2O after pretreatment with medium- versus short-chain alcohols. This suggests that the medium-chain alcohols may be more effective at extracting a lipoidal component of skin which is responsible for the resistance of skin to the permeation of polar solutes and solvents such as theophylline and PG and the back-diffusion of H_2O.

References

Behl, C.R., Flynn, G.L., Kurihara, T., Harper, N., Smith, W., Higuchi, W.I., Ho, N.F.H., Pierson, C.L., 1980. Hydration and percutaneous absorption: I. Influence of hydration on alkanol permeation through hairless mouse skin. J. Invest. Dermatol. 75, 346–352.

Cooper, E.R., 1984. Effect of substituents on benzoic acid transport across human skin in vitro. J. Control. Release 1, 153–156.

Friend, D., Catz, P., Heller, J., Reid, J., Baker, R., 1988. Transdermal delivery of levonorgestrel. I. Alkanols as permeation enhancers in vitro. J. Control. Release 7, 243–250.

Goldberg-Cettina, M., Liu, P., Nightingale, J., Kurihara-Bergstrom, T., 1995. Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alcohols. Int. J. Pharm. 114, 237–245.

Higuchi, T., 1960. Physical chemical analyses of percutaneous absorption process from creams and ointments. J. Soc. Cosmet. Chem. 11, 85–97.

Kai, T., Mak, V.H.W., Potts, R.O., Guy, R.H., 1990. Mechanism of percutaneous penetration enhancement: effect of *n*-alkanols on the permeability harrier of hairless mouse skin. J. Control. Release 12, 103–112.

Kurihara-Bergstrom, T., Flynn, G.L., Higuchi, W.I., 1986. Physicochemical study of percutaneous absorption enhancement by dimethyl sulfoxide: kinetic and thermodynamic determinants of dimethyl sulfoxide mediated mass transfer of alkanols. J. Pharm. Sci. 75, 479–486.

Ostrenga, T., Steinmetz, C., Poulsen, B., 1971. Significance of vehicle composition. I. Relationship between topical vehicle composition, skin penetrability and clinical efficacy. J. Pharm. Sci. 60, 1175–1179.

Scheuplein, R.J., Blank, I.H., 1973. Mechanism of percutaneous absorption: penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquid. J. Invest. Dermatol. 60, 286–296.

Siver, K.G., Sloan, K.B., 1988. The effect of structure of Mannich base prodrugs of 6-mercaptopurine on their ability to deliver 6-mercaptopurine through hairless mouse skin. Int. J. Pharm. 48, 195–206.

Sloan, K.B., Koch, S.A.M., Siver, K.G., Flowers, F.P., 1986. The use of solubility parameters of drug and vehicles to predict flux. J. Invest. Dermatol. 87, 244–252.

Sloan, K.B., Beall, H.D., Villanueva, R., Weimar, W.R., 1991. The effect of receptor phase composition on the permeability of hairless mouse skin in diffusion cell experiments. Int. J. Pharm. 73, 97–104.

Smith, R.N., Hansch, C., Ames, M.M., 1975. Selection of a reference partitioning system for drug design work. J. Pharm. Sci. 64, 599–606.

Zatz, J.L., Dalvi, E.R., 1983. Evaluation of solvent-skin interaction in percutaneous absorption. J. Soc. Cosmet. Chem. 34, 327–334.

.