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Influence of Azone® and lauryl alcohol on the transport of acetaminophen and ibuprofen through shed snake skin

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Summary

The influence of the penetration enhancers Azone® and lauryl alcohol on the transport of acetaminophen and ibuprofen through shed snake (*Elaphe obsoleta obsoleta*) skin has been investigated in vitro. Finite dose studies were conducted in unoccluded Franz diffusion cells to determine the extent of penetration enhancement. Infinite dose studies were conducted in Side-bi-Side® diffusion cells to determine the mechanism of enhancement. Mixed volatile/nonvolatile solution formulations were used in all experiments; the formulations contained a drug (1% w/v), an enhancer (5% w/v) and the nonvolatile solvent propylene glycol (5% w/v) dissolved in methanol. The addition of either enhancer increased the amount of acetaminophen transported. In contrast, only lauryl alcohol increased the amount of ibuprofen transported; Azone® provided no significant enhancement. For the acetaminophen/lauryl alcohol, acetaminophen/Azone® and ibuprofen/lauryl alcohol formulations, infinite dose studies showed that the mechanism of enhancement was an increase in partition coefficient, K, with no significant increase in the diffusion coefficient, D. For the Azone®/ibuprofen formulation, the enhancer provided a slight increase in D, which was offset by a decrease in K. The results highlight the importance of partitioning in penetration enhancement.

Introduction

Human skin provides a nearly impermeable barrier to the transport of most drugs. A major contribution to this barrier is provided by the outermost layer of the skin, the stratum corneum (Scheuplein and Blank, 1971). The so-called penetration enhancers have been studied as a means to reduce this barrier. Of major interest are substances which have a mild, and ideally reversible, effect on the stratum corneum and with which the properties of the stratum corneum can be altered to advantage (Scheuplein and Ross, 1970). Many substances have been studied for their penetration enhancing effects, including surfactants (Scheuplein and Ross, 1970; Sarpotdar and Zatz, 1986), fatty acids and glycols (Cooper et al., 1985; Sheth et al., 1986), urea (Beastall et al., 1986) and dipolar aprotic solvents (Akhter and Barry, 1985; Sharata and Burnette, 1988).

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In addition, Azone® (1-dodecylazacycloheptan-2-one) has been reported to enhance the penetration of drugs across the skin. Azone® has been reported to increase the diffusivity of 5-fluorouracil (Morimoto et al., 1986) through the stratum corneum of the hairless rat. Okamoto et al. (1988) report no change in the diffusion coefficient but a marked increase in the partition coefficient of 6-mercaptopurine with Azone®. Alkanols have also been reported to act as penetration enhancers (Chien et al., 1988). The mechanism of penetration enhancement by alkanols is thought to be solubilization, with the alkanol increasing the solubility of the drug in the fatty matrix of the stratum corneum. In this study, Azone® and lauryl alcohol (dodecyl alcohol) were used as penetration enhancers. Azone® was selected because it is a potent enhancer whose mechanism of action remains unclear. Lauryl alcohol was selected for comparison with Azone® because, like Azone®, it has a dodecyl side chain. In addition, Okamoto et al. (1988) have suggested that the optimal alkyl chain length for an enhancer is about 12 methylene groups, regardless of the series of compounds.

The naturally shed skin of black rat snakes (*Elaphe obsoleta obsoleta*) was used as a model membrane in these experiments. The similarities between snake skin and human stratum corneum and the use of snake skin in percutaneous absorption studies have been reported previously (Bhatt et al., 1989; Itoh et al., 1990). Briefly, although snake skin is not a mammalian integument, it has some features that make it useful as a model membrane. Snakes molt periodically. This shed skin has no living tissue, so it can be stored refrigerated for relatively long periods. Both shed snake skin (Banerjee and Mittal, 1980) and human stratum corneum (Scheuplein and Blank, 1971) are composed of keratinized proteins and lipids. Water permeation characteristics of snake skin are similar to those of human skin (Scheuplein and Ross, 1970; Roberts and Lillywhite, 1980).

The specific objectives of the current study were: (1) to determine the effects of Azone® and lauryl alcohol on the transport of acetaminophen and ibuprofen through shed snake skin, and (2) to determine the mechanisms of penetration enhancement. Solution formulations, containing mixtures of volatile and nonvolatile solvents, were used in all experiments. Solution formulations offer the advantage of minimal mass transport limitations in the vehicle. A theoretical analysis of transdermal delivery from vehicles of this type has been reported previously (Bhatt et al., 1989).

Materials and Methods

Materials

Acetaminophen, ibuprofen and lauryl alcohol were obtained from Sigma Chemicals (St. Louis, MO). HPLC grade methanol and acetonitrile, propylene glycol and ACS grade buffer components were obtained from Fisher Scientific (St. Louis, MO). Azone® was a gift of Nelson Research (Irvine, CA). Distilled deionized water was used throughout.

Test formulations

Solution formulations of the drugs and enhancers were used in all experiments. Control formulations with no enhancer contained ibuprofen or acetaminophen (1% w/v) and propylene glycol (5% w/v) dissolved in methanol. For test formulations containing an enhancer, either Azone® or lauryl alcohol (5% w/v) was also added. A mixture of propylene glycol and methanol was used as a combined volatile : nonvolatile solvent vehicle. In preliminary experiments using acetone as a pure volatile solvent vehicle, no transport of progesterone or acetaminophen across snake skin was observed (Bhatt et al., 1989). The presence of white powders on the skin surface suggested that precipitation of the drugs had occurred. A mixed volatile : nonvolatile solvent vehicle was then selected, as suggested by Coldman et al. (1969). Methanol is a suitable volatile solvent because it has a relatively high vapor pressure at room temperature, and its delipidizing action on stratum corneum lipids has been reported to be weak (Scheuplein and Ross, 1970). It is unsuitable for in vivo experiments because toxicities are associated with its percutaneous absorption. Propylene glycol was selected as the nonvolatile solvent because it has a low vapor pressure at room temperature, is miscible with

methanol and is commonly used as a solvent for pharmaceuticals.

Finite dose studies

Finite dose studies were conducted in Franz-type diffusion cells. In these cells, one side of the sample membrane is exposed to a buffer solution which is contained in the 'receptor compartment', while the other side of the membrane is exposed to the atmosphere. The cells used in these experiments had an 8.5 ml receptor compartment that was filled with pH 7.2 isotonic phosphate buffer (US Pharmacopeia XIX, 1975). The naturally shed skin of black rat snakes (*E. obsoleta obsoleta*) was used as a model of the stratum corneum. Before each experiment, the snake skin was hydrated by placing it in water at 30°C for 30 min. It was then blotted dry and placed between the donor and receptor cells. At the beginning of an experiment, 25 μl of the test formulation were placed on the skin surface with a Hamilton microsyringe (Hamilton, Reno NV). The cell assembly was then placed in a water bath maintained at 30°C. The fluid in the receptor compartment was stirred magnetically. At the end of 72 h, 200 μl of the receptor fluid were removed and analyzed for acetaminophen or ibuprofen content using the HPLC techniques described below. During the course of these experiments, the solution formulation on the skin surface did not remain clear and homogeneous, suggesting some precipitation of the drug and/or enhancer following evaporation of the volatile solvent.

Infinite dose studies

Infinite dose studies were carried out using Side-bi-Side® diffusion cells (Crown Glass, Somerville, NJ) thermostatted to 30°C. The diffusional surface area of this cell is 0.785 cm^2 and the volumes of the donor and receptor compartments were each 3.4 ml. The donor compartment contained the test formulation of the drug and enhancer in the volatile : nonvolatile solvent vehicle described above. The receptor compartment contained isotonic pH 7.2 phosphate buffer. These donor and receptor solutions were selected to correspond to the conditions in the finite dose studies, and to ensure that the drug and enhancer remained in solution in the donor compartment. Before each experiment, the snake skin was hydrated by placing it in water at 30°C for 30 min, as in the finite dose studies. During the experiments, 200 μl samples of the receptor fluid were withdrawn at regular intervals and assayed for acetaminophen or ibuprofen using the HPLC techniques described below. The volume of fluid withdrawn was replaced with fresh buffer, and the data were corrected for the drug mass removed.

The data obtained from the infinite dose experiments were plotted as the cumulative amount of drug appearing in the receptor compartment as a function of time. Typically, these plots displayed an initial lag-phase, during which no drug appeared in the receptor compartment, followed by a phase in which the cumulative amount transported was linearly related to time. This profile is consistent with steady-state Fickian diffusion through a membrane following a lag-phase. Accordingly, the data were analyzed using the appropriate equations for Fickian diffusion. Regression of the linear (steady-state) portion of the plots gives an intercept on the abscissa. The diffusion coefficient, D, of the drug in the membrane is related to the x-intercept by the equation (Crank, 1985):

$$x\text{-intercept} = h^2/6D \quad (1)$$

where h is the membrane thickness. The slope of the linear portion of the plot is proportional to the membrane permeability, P, and is related to both the diffusion coefficient and the partition coefficient, K, by the equation (Crank, 1985):

$$\text{slope} = PC_d = (DKC_d)/h \quad (2)$$

where C_d is the concentration of the drug in the the donor compartment and K is the partition coefficient of the drug from the donor solution into the snake skin membrane (C_{memb}/C_{soln}).

Measurement of skin thickness

In order to calculate effective diffusion coefficients from permeability data, the skin thickness must be known. Samples of shed snake skin were

fixed, dehydrated in an alcohol series and embedded in Spurr low-viscosity embedding medium. Thin cross-sections of the embedded material were cut with a microtome and viewed in a Philips 510 scanning electron microscope to determine the thickness of the samples. The mean thickness was 0.0010 ± 0.0002 cm ($n = 4$).

Analytical methods

High-performance liquid chromatography (HPLC) was performed with a Perkin Elmer (Norwalk, CT) system consisting of a series 410 LC pump, an ISS-100 autoinjector, an LC 90 UV variable-wavelength spectrophotometric detector and an LCI-100 laboratory computing integrator. The columns used for HPLC were supplied by Brownlee Labs (Santa Clara, CA).

In the reversed-phase assay for ibuprofen, a silica guard column (4.6 × 30 mm) and an ODS analytical column (4.6 × 100 mm) were used. The mobile phase consisted of acetonitrile (50% v/v) in pH 3.0 buffer (0.01 M NaH_2PO_4 solution adjusted to pH 3.0 with 0.01 M H_3PO_4 solution). Under these conditions, the retention volume of ibuprofen was 4.1 ml. Ibuprofen was quantitated by measuring the peak areas at 220 nm. The concentration in the analyte solution was determined by comparison with a standard curve prepared using ibuprofen solutions of known concentration. The correlation coefficient for the standard curve was greater than 0.999. Peak area was linearly related to concentration for samples containing 7.5–2500 ng of ibuprofen injected onto the column.

Acetaminophen was also analyzed using a reversed-phase HPLC procedure. A guard column (4.6 × 30 mm) and an analytical column (4.6 × 100 mm) were both packed with ODS Spheri-5 particles. A mobile phase system of methanol (20% v/v) in water was used to obtain a retention volume for acetaminophen of 3 ml. Acetaminophen was quantitated by measuring peak areas at 248 nm. The concentration in the analyte solution was determined by comparison with a standard curve prepared using acetaminophen solutions of known concentration. The correlation coefficient for the standard curve was greater than 0.999. Peak area was linearly related to concentration for 20–1000 ng of acetaminophen injected onto the column.

Results and Discussion

Results of the finite dose experiments are shown in Fig. 1. Over the 72 h period of the experiment, lauryl alcohol provided a 1.9-fold increase in the amount of ibuprofen transported and a 5.5-fold increase in the amount of acetaminophen transported relative to a control formulation containing no enhancer. Similarly, Azone® provided a 4.7-fold increase in the amount of acetaminophen transported relative to control. However, there was no significant enhancement of ibuprofen transport when Azone® was used as a penetration enhancer.

The infinite dose studies provide some insight into these observations; the results of these studies are shown in Fig. 2. The figure shows the time course of the permeation of acetaminophen or ibuprofen from formulations with no enhancer (panels a and d), with lauryl alcohol as an enhancer (b and e) or with Azone® as an enhancer (c and f). In the figures, each symbol represents a different experiment. The slopes and x-intercepts of the individual experiments were used to esti-

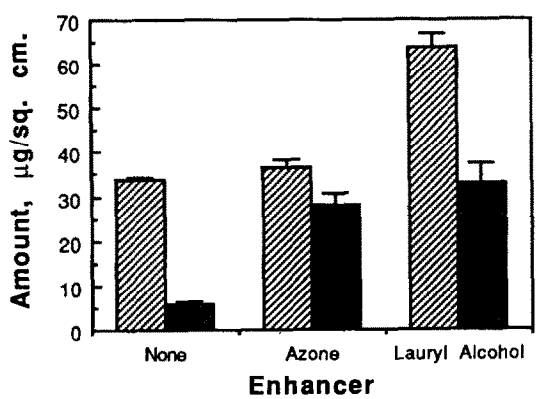

Fig. 1. Effect of penetration enhancers on the cumulative amount of ibuprofen (▨) or acetaminophen (■) transported through shed snake skin in 72 h. Experiments were performed in Franz-type diffusion cells using solution formulations of the drugs (drug (1% w/v), enhancer (5% w/v) and propylene glycol (5% w/v) dissolved in methanol). Error bars represent standard deviations; $n = 6$.

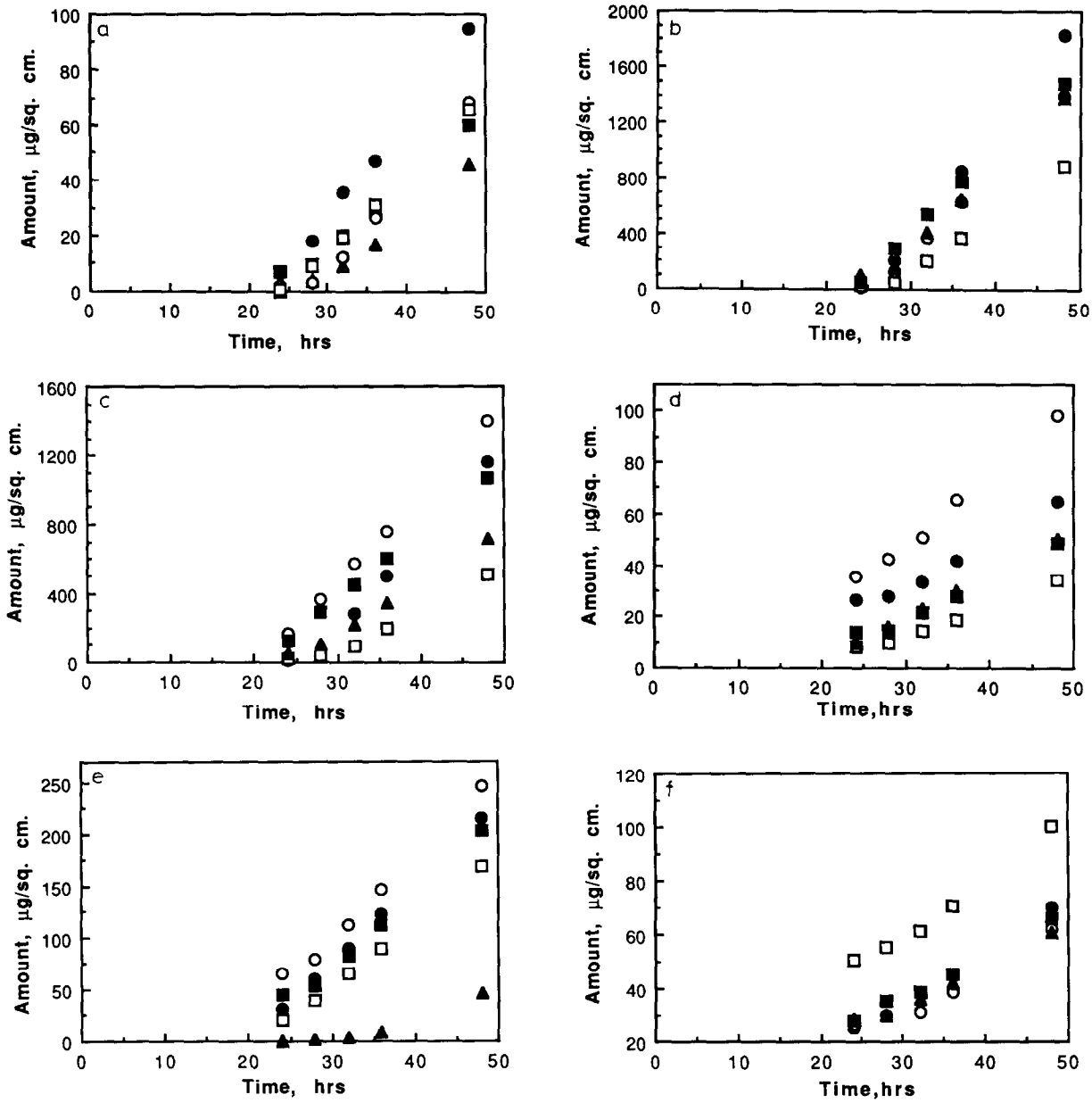

Fig. 2. Results of the infinite dose studies, showing the cumulative amount of acetaminophen or ibuprofen transported through shed snake skin as a function of time from various formulations. Each symbol represents a single experiment. Experiments were performed in Side-bi-Side® diffusion cells using a solution formulation of the drug in the donor compartment (drug (1% w/v), enhancer (5% w/v) and propylene glycol (5% w/v) dissolved in methanol) and pH 7.2 phosphate buffer in the receptor compartment. The formulations were: (a) acetaminophen with no enhancer, (b) acetaminophen with lauryl alcohol as enhancer, (c) acetaminophen with Azone® as enhancer, (d) ibuprofen with no enhancer, (e) ibuprofen with lauryl alcohol as enhancer, (f) ibuprofen with Azone® as enhancer.

mate the diffusion and partition coefficient values according to Eqns 1 and 2. In all the infinite dose experiments, the standard errors in the slopes averaged 26% of the slope value, with a range from 10 to 32%. The standard errors in the x-intercepts averaged 18% of the intercept value, with a range of 6–34%.

The diffusion and partition coefficient values determined in the infinite dose experiments are summarized in Table 1. Each reported value is the mean of the five individual values of Fig. 2. When lauryl alcohol was used as an enhancer, the partition coefficients of both ibuprofen and acetaminophen were increased significantly relative to the control formulation. However, the diffusion coefficients either decreased slightly (ibuprofen) or were unaffected (acetaminophen). For these formulations, the apparent mode of penetration enhancement by lauryl alcohol is an increase in drug partitioning into the membrane. Similar results were observed with acetaminophen when Azone® was used as a penetration enhancer: there was a dramatic increase in the partition coefficient, with no significant change in the diffusion coefficient. However, when Azone® was used as a penetration enhancer for ibuprofen, there was a significant increase in the diffusion coefficient, but a marked decrease in the partition coefficient. As a result of these counterbalancing effects, the permeability (see Eqn 2) of ibuprofen in the presence of Azone®, 5.8 (± 1.7) $\times 10^4$ cm/s, is not significantly different from its permeability in the control formulation without enhancer, 5.2 (± 1.2) $\times 10^4$ cm/s. The increase in the diffusion coefficient is offset by a decrease in the partition coefficient, so that no net enhancement is observed. These results are consistent with the lack of penetration enhancement observed with this formulation in the finite dose experiments for Azone® (Fig. 1).

There are certain limitations inherent in the theoretical and experimental approaches employed here. First, diffusion coefficients determined from lag-times are not generally considered to be as accurate as those determined by other methods. In addition, the 'diffusion coefficient' for snake skin must be regarded as an effective value, since the membrane itself is a heterogeneous layer consisting of protein and lipid regions. Furthermore, the diffusion and partition coefficients reported are specific to the solutions used; since the donor and receptor compartment solvents are not identical, differences in solute activity in these two solvent systems are expected to influence the D and K values. The Fickian analysis also presumes that the diffusion and partition coefficients are constant throughout the experiment. Specifically, it is assumed that the drug, enhancer and vehicle effects on drug diffusivity and partitioning are exerted so rapidly (or so slowly) that the values are effectively constant. The observed linearity of the steady-state portion of plots of the cumulative mass transported as a function of time (Fig. 2) suggests minimal changes in the coefficients during this time period. How-

TABLE 1

Effect of penetration enhancers on the diffusion (D) and partition (K) coefficients of ibuprofen and acetaminophen in shed snake skin [a]

Enhancer	Ibuprofen		Acetaminophen	
	D (cm^2/s) ($\times 10^{12}$) ($\pm$S.D.) [b]	K ($\pm$S.D.) [b]	D (cm^2/s) ($\times 10^{12}$) ($\pm$S.D.) [b]	K ($\pm$S.D.) [b]
None	2.8 (0.5)	19 (3)	1.8 (0.2)	46 (10)
Azone®	4.7 * (1.5)	12 * (5)	1.9 (0.3)	580 * (190)
Lauryl alcohol	2.1 * (0.4)	85 * (13)	1.8 (0.1)	930 * (210)

[a] Measured in Side-bi-Side® diffusion cells. Donor compartment contained drug (1% w/v), propylene glycol (5% w/v) and enhancer (5% w/v) in methanol. Receptor compartment contained pH 7.2 phosphate buffer.

[b] Standard deviation ($n = 5$).

* Significantly different from the formulation with no enhancer at 99% confidence ($P < 0.01$) by Student's t-test.

ever, it is impossible to determine from the present data whether changes occur during the lag phase. In a previous study, diffusion coefficient values determined from lag times differed by less than a factor of five from values determined by fitting experimental data to a mathematical model for delivery from a solution vehicle (Bhatt et al., 1989). The formulation used in that study was identical to the acetaminophen formulation used here; snake skin was also used as the model membrane. The reasonably good agreement between diffusion coefficients determined by the two methods is encouraging, and lends support to the use of the lag-time method in the present study.

The partition coefficient values are also affected by errors in the measurement of the skin thickness, h. The dehydrated skin thickness was measured in these experiments, but the hydrated film thickness is the relevant value for transport studies. The partition coefficient values are therefore in error by the ratio of the hydrated film thickness to the dehydrated film thickness. This is expected to be a systematic error, affecting all the partition coefficient values roughly equally.

With these limitations, the partition and diffusion coefficient values should be regarded as approximate measurements. Nevertheless, some conclusions about the mechanisms of penetration enhancement can be made. The mechanism of penetration enhancement for lauryl alcohol in both ibuprofen and acetaminophen formulations appears to be improved partitioning of the drug into the skin. Improved partitioning is also the mechanism of enhancement for Azone® in the acetaminophen formulation. In these cases, the effect of the enhancer on the diffusion coefficient is minimal. A slight increase in diffusion coefficient was observed when Azone® was added to the ibuprofen formulation; this increase was offset by a decrease in the partition coefficient. As a result, no penetration enhancement was observed.

In the formulations studied here, then, the 'penetration enhancers' are acting as 'partitioning enhancers' when they are effective. It is interesting to note that the enhancers increased the value of the partition coefficient by as much as a factor of twenty. It is unlikely that such large increases in the diffusion coefficient could be achieved without damage to the membrane. In the development of new penetration enhancers, it may be useful to focus on their partitioning enhancement abilities.

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