Pharmaceutical Research, Vol. 7, No. 7, 1990

Report

Percutaneous Absorption Enhancement of an Ionic Molecule by Ethanol–Water Systems in Human Skin

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Received June 26, 1989; accepted January 26, 1990

Ethanol–water systems enhance permeation of ionic solutes through human stratum corneum. Optimum enhancement of salicylate ion permeation has been observed with ethanol volume fractions near 0.63. The mechanism of action of ethanol–water systems enhancing skin permeation was investigated by *in vitro* skin permeation studies combined with Fourier transform infrared spectroscopy experiments. The increased skin permeation of the ionic permeant by the ethanol–water systems may be associated with alterations involving the polar pathway. Polar pathway alterations may occur in either or both the lipid polar head and proteinaceous regions of the stratum corneum. Ion-pair formation may also contribute to increased permeation. However, the decreased permeation of salicylate ion observed at higher volume fractions of ethanol may be attributed to decreased uptake of permeant into the stratum corneum.

KEY WORDS: transdermal enhancement; percutaneous absorption; transdermal permeation; stratum corneum; penetration enhancement.

INTRODUCTION

Transdermal drug delivery offers certain advantages over conventional routes of drug administration. However, barrier properties of intact adult skin (1,2) limit the permeation of a wide variety of substances, including pharmaceutically effective agents. Therefore, only a few drug molecules having optimal physicochemical properties penetrate the skin sufficiently to be therapeutically effective. One approach to overcome these constraints is to incorporate solvents into the vehicle that alter the thermodynamic and kinetic limits of the membrane and drug so as to enhance transport through the skin (3–6).

Ethanol is a known flux enhancer for the transdermal delivery of relatively lipophilic drugs (7–9). The increased transdermal flux of a highly lipophilic drug, β-estradiol, in the presence of ethanol resulted in β-estradiol fluxes within clinically useful ranges (8). *In vitro* human epidermal flux from a 0.70-volume fraction ethanol–water mixture saturated with β-estradiol was reported to be approximately 20 times greater than that found for saturated aqueous solutions. Recently, the effects of ethanol on the transport of β-estradiol and other permeants through hairless mouse skin were investigated over the 0.0- to 1.0-volume fraction ethanol–saline range (10,11). It was hypothesized that the ethanol vehicle altered the lipoidal pathway primarily below ethanol–saline fractions of 0.30, while the polar pathway was significantly affected at higher ethanol volume fractions.

The stratum corneum, which is composed of desquamated keratinized cells imbedded in a multilayer lipid matrix, is often the rate-limiting barrier to transdermal drug delivery for most compounds. Polar solutes are hypothesized to diffuse through the stratum corneum via a polar pathway. The exact nature of the pathway is unknown, although it is likely to involve either or both the keratinized protein cell remnants and the polar head regions of the lipid domain.

The keratinized proteins in stratum corneum are similar to the highly ordered keratin fibers in hair and wool, only stratum corneum proteins have considerably fewer half-cystine amino acid residues. As a result, stratum corneum keratinized proteins are less extensively cross-linked by disulfide bonds (12–14). X-ray diffraction studies using short-chain alcohols (methanol, ethanol, *n*-propanol, and *n*-butanol) have shown that these solvents swell the crystalline regions of the microfibrils in the α-keratin fibers of hair and wool (15).

Recent studies in model phospholipid membrane systems (16–18) have demonstrated a biphasic transition behavior in the presence of short-chain alcohols. Low alcohol concentrations were associated with a decrease in the main gel-to-liquid crystalline phase transition temperature, while higher concentrations resulted in an increase in the transition temperature. Rowe (17) concluded that low concentrations

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0724-8741/90/0700-0762$06.00/0

act preferentially on the fluid regions of the bilayer. At higher concentrations, ethanol was suggested to stabilize the gel phase. The reversal of the transition temperature with increasing ethanol concentrations has been proposed to result from the interdigitation of the lipid bilayers (18). Interdigitation was hypothesized to result from solvent replacement of some of the waters of hydration surrounding the polar head groups, thus increasing the surface area of the interfacial polar head region. Interdigitation has also been speculated to occur within minor regions of the hairless mouse stratum corneum lipid domains in the presence of low concentrations of short-chain alcohols (19). Similar types of lipid polar head group reorganization in human stratum corneum could increase the effective volume within the hydrated head group region of the lipid multilayers that is available for transport.

In addition, electrolytes in ethanol–water mixtures may form ion pairs (20–23). The conductivity of chlorpromazine, sodium salicylate, and calcium saccharide in aqueous ethanol solutions decreased as the ethanol concentration increased (23). The dissociation constants of ion pairs decrease as the dielectric constant of the medium decreases. Thus, the conductivity decrease suggests an increase in the extent of ion-pair formation as a function of increasing ethanol.

The role of the ethanol–water system in the percutaneous absorption of a polar permeant, salicylate ion, has been investigated with classical diffusion studies in combination with spectroscopic studies to understand ethanol-induced alterations of the stratum corneum barrier. Ethanol–water systems may be useful in the development of transdermal drug delivery systems, particularly for the formulation of ionic drugs which may not have optimal flux values.

EXPERIMENTAL METHODS

Materials

Human cadaver skin (back area) was obtained from a local skin bank and stored at −80°C until used for permeation studies or prepared for spectroscopic studies.

Immediately prior to a diffusion experiment, whole skin was thawed in pH 7.4 phosphate-buffered saline (PBS) at 32°C for at least 30 min. Intact epidermis was isolated from dermis using the heat separation method (1). Thawed whole skin was immersed in PBS solution for exactly 2 min at 60°C. The epidermis was carefully peeled away from the dermis and used for the diffusion experiment.

Stratum corneum sheets were isolated from unused epidermal sheets by trypsin digestion. The epidermis was incubated in phosphate-buffered saline (PBS; pH 7.4) containing 0.25% trypsin (T360 Purified, Fisher Chemical, Pittsburgh, PA) and 0.01% gentamicin (Sigma Chemical Co., St. Louis, MO) at 32°C for 24 hr. The isolated stratum corneum was rinsed with saline and stored on Teflon sheets to be dried under vacuum overnight at room temperature and stored desiccated until used for spectroscopic studies.

Sodium salicylate and ethanol were purchased from Aldrich Chemical Company (Milwaukee, WI) and USI Chemicals (Tuscola, IL), respectively. Perdeuterated ethanol (EtOD) and deuterium oxide (D_2O) were obtained from Sigma Chemicals.

In Vitro Permeation Studies

Vertically assembled diffusion cells with an effective diffusional area of approximately 2 cm^2 and a downstream volume of 10 ml were used. Each cell was individually calibrated with respect to its receiver volume and diffusional surface area. A Teflon stirrer was used only in the receiver compartment and was driven by a constant-speed motor.

The receiver compartment was filled with saline containing 0.01% gentamicin. Human epidermis was then mounted on the cell, stratum corneum side up, and the donor compartment cap was clamped in place. At zero time, the donor compartment was charged with 3 ml ethanol–water solution (between 0.00 and 1.00 volume fraction of ethanol, V_e) of known concentration of salicylate ion at pH 7.0. For $V_e < 0.80$, salicylate ion concentrations were 250 mg/ml in ethanol–water donor solutions. At 0.80 and 1.00 V_e, the donor solutions were saturated with sodium salicylate (50 and 26 mg/ml, respectively). The experiments were performed at 32°C for 60 hr. Two hundred-microliter aliquots were withdrawn from the receiver compartments periodically and replaced with 200 μl of saline solution. The samples were diluted when necessary and assayed for salicylate ion and ethanol concentrations.

Salicylate ion concentrations were assayed by high-performance liquid chromatography (HPLC) using a C_{18} Novapac column with a flow rate of 1.0 ml/min of a mobile phase consisting of 65/35 (v/v) 0.005 *M* acetic acid/methanol at pH 4.0. The retention time of salicylate ion was 2.8 min. Sample detection was accomplished by UV absorption at 230 nm.

Ethanol concentrations were assayed using gas chromatography (GC). A 0.2% Carbowax 1500 on Carbopak C 60/80-mesh column with a nitrogen flow rate of 15 ml/min was used. Sample detection was accomplished by flame ionization. Column, injection port, and detector temperatures were 110, 180, and 200°C, respectively. The retention time for ethanol was just over 1.3 min.

Simultaneous steady-state fluxes (J^{ss}) of salicylate ion and ethanol across the membranes were calculated using the equation

$$J^{ss} = [V_R/A]dC_R/dt \quad (1)$$

where V_R is the receiver volume, dC_R/dt is the steady-state rate of change in concentration in the receiver compartment (obtained from the slope of a linear least-squares fit of the cumulative amount penetrated versus time), and A is the diffusional area. Steady-state fluxes of salicylate ion and ethanol were achieved within 14 hr for $V_e < 0.80$ and approximately 40 hr for $V_e \geq 0.80$. The permeability coefficients were determined by

$$P = J^{ss}/C_D \quad (2)$$

where P is the permeability coefficient and C_D is the initial salicylate ion or ethanol concentration in the donor chamber solution. The experiments were carried out in a manner which allowed use of the donor concentration for the concentration differential.

Uptake Studies

The uptake of sodium salicylate into the stratum corneum was determined by placing two 1.325-cm^2 disks of intact, desiccated stratum corneum from the corresponding skin donor in 1 ml of each donor solution. After 48 hr of incubation at 32°C (110 rpm), the disks were removed from the solutions and thicknesses measured. Disks were dipped twice in 50/50 ethanol–water solutions to remove any surface-adsorbed sodium salicylate. The disks were then extracted for 96 hr in 50/50 ethanol–water at 32°C, and the extraction media were exchanged for fresh solutions at 72 hr. The combined extracts were diluted to 10 ml and assayed by HPLC as previously described to determine the concentration of sodium salicylate in the stratum corneum. The data were calculated as the uptake of sodium salicylate (mg) per cubic centimeter of hydrated stratum corneum in the aqueous ethanol solutions.

Fourier Transform Infrared Spectroscopy (FTIR) Studies

In order to examine stratum corneum structure under conditions similar to those of the permeation experiments, the stored, desiccated stratum corneum samples were rehydrated at room temperature by suspension in a chamber containing liquid deuterium oxide in equilibrium with vapor for 22 to 25.5 hr. The samples were then immersed in the appropriate EtOD–D_2O solution for 6 hr. Deuterium shifts vibrational modes of solvent origin away from those associated with stratum corneum. Deuterium will also replace exchangeable hydrogen atoms, thus shifting the associated vibrational modes to other spectral regions. Deuterium–hydrogen exchange and deuterium effects on secondary interactions do not affect the protein Amide I (C=0 stretching) vibrational modes which provide information concerning protein conformation; however, the Amide II (N–H bending and C–N stretching) and N–H stretching vibrational modes, useful for determining lipid extraction, are affected by the exchange (24–26). Samples were handled under a nitrogen atmosphere to prevent deuterium exchange with atmospheric hydrogen. The samples were then sealed between two zinc selenide disks and placed in a Barnes–SpectraTech (Stamford, CT) temperature cell.

To investigate possible lipid extraction, another series of samples was prepared in a similar procedure using nondeuterated ethanol and water. Nondeuterated solvent mixtures were studied to avoid spectral artifacts associated with deuterium exchange. The samples were vacuum-dried for 16 hr at room temperature and stored desiccated to remove overlapping water and ethanol O–H stretching vibrational models in the 3300-cm^{-1} region.

Transmission FTIR studies of the stratum corneum sheets were performed with a Digilab FTS 20-80 (Cambridge, MA) Fourier transform infrared spectrometer equipped with a liquid nitrogen-cooled, narrow-band MCT detector. Spectra (1.0-cm^{-1} resolution, 256 scans, noise level of 0.1, sensitivity of 1, zero-fill factor of 2, and triangularly apodized) were obtained over the midinfrared region from 3900 to 900 cm^{-1}. Isothermal conditions (32 ± 0.25°C) were maintained with an Omega (Stamford, CT) temperature controller during data collection. The data presented are representative of three samples which all behaved similarly.

Spectral manipulations were performed with SpectraCalc (Galactic Industries, Salem, NH) and Digilab data manipulation packages. The individual spectra were baseline corrected to give a baseline at zero ($\leq \pm 0.01$ absorbance unit) prior to further data manipulation.

RESULTS AND DISCUSSION

The transport behavior of salicylate ion and ethanol across human epidermis was investigated as a function of the volume fraction of ethanol (V_e). The steady-state permeability coefficients of salicylate ion and ethanol are presented in Figs. 1 and 2, respectively. With increasing ethanol concentration, the permeability coefficient of salicylate ion is constant at 1.5×10^{-8} cm/sec within experimental error for $0.00 \leq V_e \leq 0.20$. The permeation of another ionic solute, tetraethylammonium bromide, through hairless mouse skin was also observed to remain constant for aqueous ethanol mixtures equal to or less than 0.25 V_e (11). The permeation of salicylate ion then increases for donor solutions above 0.20 V_e. Salicylate ion permeation reaches a maximum near 0.63 V_e, where the permeability coefficient is $7.03 (\pm 2.6) \times 10^{-8}$ cm/sec, approximately five times greater than that found for aqueous solutions. The permeation then decreases with increasing ethanol concentrations in the donor chamber. The solubility of salicylate ion decreased from greater than 250 mg/ml ($V_e < 0.80$) at pH 7.0 to 26 mg/ml ($V_e = 1.00$) in the ethanol–water solutions.

The ethanol permeability coefficient through epidermis in the presence of salicylate ion increases with increasing ethanol concentration, maximizes near 0.63 V_e, and decreases by 1.00 V_e, as presented in Fig. 2. The permeability coefficient of ethanol at 0.63 V_e is $1.79 (\pm 0.26) \times 10^{-6}$ cm/sec. Ethanol flux through human epidermis from neat ethanol agrees well with that obtained by Scheuplein (1).

The uptake of sodium salicylate into the stratum corneum was determined as a function of the volume fraction of ethanol (V_e) as given in Table I. The sodium salicylate uptake is within the range of 157–173 mg/cm^3 stratum corneum for $0.00 \leq V_e \leq 0.63$. At $V_e = 0.95$ and 1.00, the uptake decreases dramatically to 35.4 and 8.9 mg/cm^3 stratum corneum, respectively.

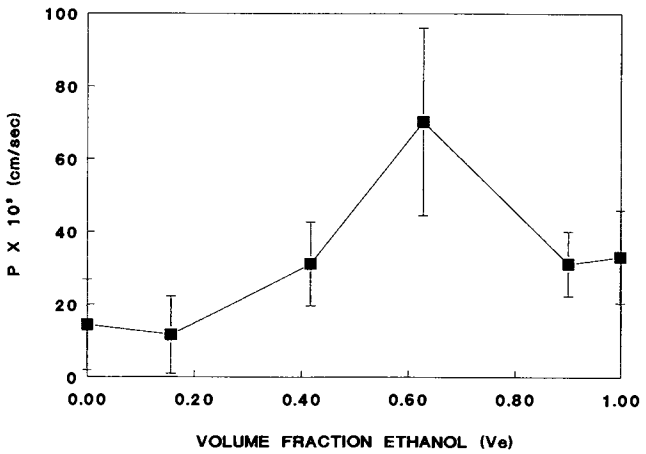

Fig. 1. Permeability coefficients of salicylate ion across human epidermis as a function of the volume fraction of ethanol.

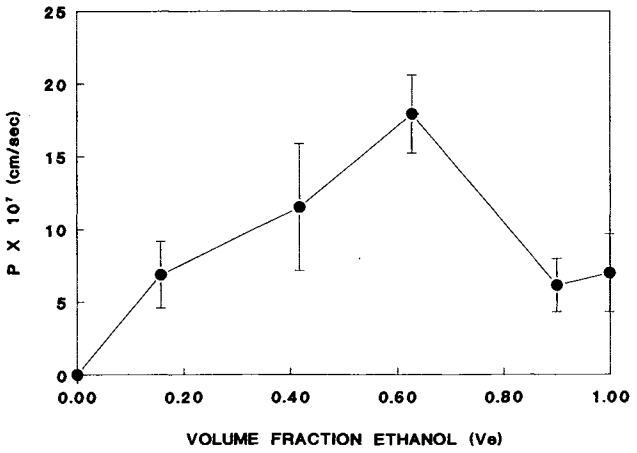

Fig. 2. Permeability coefficients of ethanol in the presence of salicylate ion across human epidermis as a function of the volume fraction of ethanol.

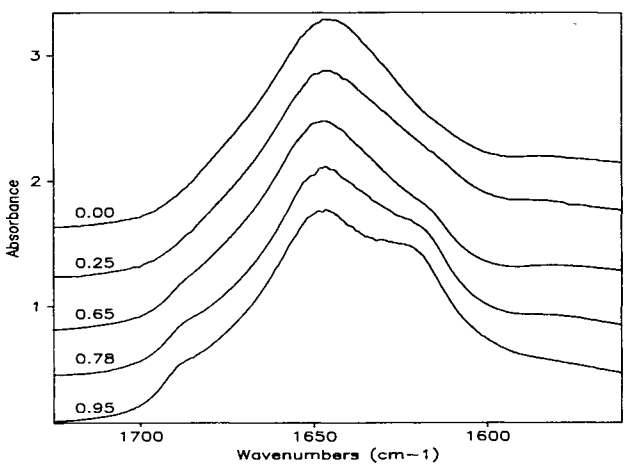

Fig. 3. Spectra of hydrated stratum corneum in the Amide I region (1750–1550 cm^{-1}) showing changes in protein conformation in the presence of 0.00, 0.25, 0.65, 0.78, and 0.95 V_e.

To investigate possible mechanisms for the increased salicylate ion fluxes associated with ethanol–water systems, the effects of ethanol on human stratum corneum have been studied by FTIR. Biophysical structural alterations within the protein domain as well as lipid extraction were determined.

The effects of ethanol on the stratum corneum membrane were determined in stratum corneum samples equilibrated with perdeuterated ethanol (EtOD) and deuterium oxide (D_2O) solutions of varying V_e. Absorption in the Amide I region near 1654 cm^{-1} has been assigned to the α-helical conformation of keratinized proteins, while absorptions near 1620 and 1690 cm^{-1} are characteristic of an antiparallel β-pleated sheet conformation (27). Similar band positions were noted for antiparallel-chain pleated sheet and extended chain conformations of polypeptides and globular proteins, respectively (28,29). When treated with isopropanol, β-globular proteins undergo a conformational change giving rise to bands near 1686 and 1616 cm^{-1} which are probably due to the formation of distorted β-strands within the proteins (25). The Amide I region of the human stratum corneum in the presence of EtOD-D_2O mixtures at 32°C is shown in Fig. 3. The keratinized protein component of the stratum corneum is predominantly α-helical, with some random coil (Amide I band centered at 1650 cm^{-1}) in the presence of perdeuterated ethanol fractions up to 0.25 V_e. As ethanol concentration is increased above 0.25 V_e, shoulders form on each side of the Amide I band near 1688 and 1615 cm^{-1}, suggesting the formation of extended chains or distorted β-strands within the protein domain.

A qualitative evaluation of lipid extraction because of ethanol pretreatment is made by comparing the intensities of infrared bands arising primarily from stratum corneum proteins to those associated with the lipid domains. Previous thermal perturbation spectroscopy studies (30,31) have assigned the N–H stretching vibrations which absorb near 3300 cm^{-1} to the protein component. The C–H stretching vibrations of stratum corneum (2950–2850 cm^{-1}) arise primarily from the alkyl chains of the lipids within the intercellular spaces. A decreased absorbance of the C–H stretching bands relative to the N–H stretching band would indicate that some degree of lipid extraction has occurred. The desiccated spectra (Fig. 4) suggest that some lipids were extracted as ethanol concentration increased. The absorbance ratio of the N–H stretching band (3320 cm^{-1}) to the asymmetric CH_2 stretching band (2917 cm^{-1}) decreased from 0.96 to 0.76 as the V_e increased from 0.40 to 1.00, respectively. A similar degree of lipid extraction is likely to occur during *in vitro* permeation studies.

Table I. Uptake of Sodium Salicylate into the Human Stratum Corneum as a Function of the Volume Fraction of Ethanol

V_e	Sodium salicylate uptake (mg/cm^3 stratum corneum)
0.00	173 ± 20[a]
0.20	159 ± 37
0.40	164 ± 39
0.63	157 ± 8
0.95	35 ± 8
1.00	8 ± 2

[a] Average ± standard deviation.

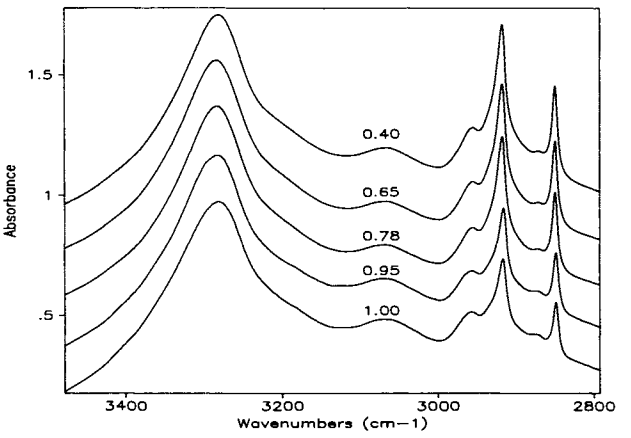

Fig. 4. Spectra of desiccated stratum corneum (3500–2800 cm^{-1}) showing lipid extraction in samples pretreated with 0.40, 0.65, 0.78, 0.95, and 1.0 V_e.

Therefore, swelling and secondary conformational alterations within the keratinized protein fibrils could possibly increase diffusional volume within the stratum corneum protein domain. Lipid extraction at high ethanol concentrations may occur in conjunction with and/or independent of conformational alterations within the protein domains. Extraction of lipids could result in a reorganization of the lipid domains. Such alterations may lead to a compromise of stratum corneum barrier function.

Salicylate ion permeation reaches a maximum near 0.63 V_e and then apparently decreases as the ethanol concentration is further increased above 0.63 V_e. Altered or additional polar pathways formed with increasing ethanol concentrations may result from a combination of alterations in protein conformation, reorganization within the lipid polar head regions, or lipid extraction. Such alterations within the polar pathway may increase salicylate ion permeation. In addition, the possible formation of salicylate ion pairs may further increase permeation as the ethanol concentration is increased in the donor solutions. At $V_e \geq 0.75$, the uptake of salicylate ion into the stratum corneum from aqueous ethanol mixtures decreased significantly (Table I). Therefore, although polar pathway or permeant alterations may occur with increasing ethanol concentrations, the decreased solubility of salicylate ion in the stratum corneum may limit its ability to take advantage of these alterations. The competing processes of structural alteration within the polar pathway, ion-pair formation, and decreased salicylate ion solubility in the stratum corneum result in optimal salicylate ion permeation near 0.63 V_e.

CONCLUSIONS

The mechanism(s) of enhanced skin permeation of ionic compounds by ethanol–water systems may involve contributions from alteration of the stratum corneum keratinized protein conformation, the extended hydrophilic domain between the lipid polar head groups, or lipid extraction. Such morphological changes could form additional free volume within the stratum corneum, thus reducing diffusional resistance of ionic solute permeation through the stratum corneum. In addition, the solubility of the compound in the stratum corneum must be of sufficient magnitude in order to take advantage of the altered pathway.

ACKNOWLEDGMENT

The authors are grateful to Dr. William R. Good for his valuable insight and suggestions.

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