Report

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

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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 bifasic 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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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 chloropromazine, sodium salicylate, and calcium saccharide in aqueous ethanol solutions decreased as the ethanol concentration increased (23). The dissociation constants of ion pairs decreased 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 2 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 (D₂O) were obtained from Sigma Chemicals.

**In Vitro Permeation Studies**

Vertically assembled diffusion cells with an effective diffusional area of approximately 2 cm² 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, Vₑ) of known concentration of salicylate ion at pH 7.0. For Vₑ < 0.80, salicylate ion concentrations were 250 mg/ml in ethanol–water donor solutions. At 0.80 and 1.00 Vₑ, 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 C₁₈ 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 (Fₑ) of salicylate ion and ethanol across the membranes were calculated using the equation

$$Jₑ = \frac{[Vᵦ]}{A}dCₑ/dt$$

where Vᵦ is the receiver volume, dCₑ/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 Vₑ < 0.80 and approximately 40 hr for Vₑ ≥ 0.80. The permeability coefficients were determined by

$$P = \frac{Fₑ}{Cᵦ}$$

where P is the permeability coefficient and Cᵦ 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.