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Enhancing effect of alpha-hydroxyacids on "in vitro" permeation across the human skin of compounds with different lipophilicity

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Abstract

The percutaneous penetration-enhancing effects of glycolic acid, lactic acid and sodium lauryl sulphate through the human epidermis was investigated using 5-fluorouracil as a hydrophilic model permeant and three compounds belonging to the phenylalcohols: 2-phenyl-ethanol, 4-phenyl-butanol and 5-phenyl-pentanol. The lipophilicity values of the compounds ranged from $\log P_{\text{oct}}$ −0.95 to 2.89. The effect of the enhancer concentration was also studied. Skin pretreatment with aqueous solutions of the three enhancers did not increase the permeability coefficient of the most lipophilic compound ($\log P_{\text{oct}} = 2.89$). For the other compounds assayed, the increase in the permeability coefficients depended on the concentration used in skin pretreatment, and on the lipophilicity of the compounds tested—and was always greater for the most hydrophilic compound (5-fluorouracil), for which lactic acid exerted a greater enhancer effect than glycolic acid or sodium lauryl sulphate. Primary irritation testing of the three enhancers was also carried out at the two concentrations used in skin pretreatment for diffusional experiments (1% and 5%, w/w). The least irritant capacity corresponded to lactic acid; consequently, this alpha-hydroxyacid could be proposed as a percutaneous penetration enhancer for hydrophilic molecules that are of interest for transdermal administration.

Keywords: Alpha-hydroxyacids; Sodium lauryl sulphate; Percutaneous enhancers; Lipophilicity; Skin irritation

1. Introduction

Alpha-hydroxyacids (AHAs) and sodium lauryl sulphate have been extensively used in cosmetic and dermatologic formulations. Recently, alpha-hydroxyacids have been recognized as important adjunctive therapeutic elements in a variety of skin disorders including photodamage (Funasaka et al., 2001), actinic damage, melasma (Usuki et al., 2003), hyperpigmentation (Tung et al., 2000) and acne (Atzori et al., 1999). Several studies have investigated structural and functional changes in the epidermal barrier promoted by AHAs, and their effects on skin permeability. When glycolic acid was used at low concentration (2–5%) in the volar forearm of human volunteers, electron microscopy revealed no ultrastructural changes in the nucleated layers of the epidermis, and no changes in transepidermal water loss (TEWL) were recorded (Fartasch et al., 1997). Nevertheless, repeated use of AHA formulations has been demonstrated to alter the structure of the stratum corneum (Leyden et al., 1995), viable epidermis and dermis (Lavker et al., 1992), and may result in skin barrier alterations that could cause changes in the percutaneous absorption of topically applied chemicals. In fact, Kraeling and Bronaugh (1997) showed that permeability of the skin to tritiated water increased by a factor of 2 after treatment with glycolic acid. In this context, it has been suggested that AHAs can reduce stratum corneum corneocyte cohesion through interference with ionic bonding (Kraeling and Bronaugh, 1999). Also, the permeability coefficient of ibuprofen lysine was found to be increased approximately 20 times by lactic acid (Sebastiani et al., 2005). On the other hand, sodium lauryl sulphate skin interactions have been analyzed in many studies (Lodén, 1990; Froebe et al., 1990; Leveque et al., 1993; Ribaud et al., 1994), and its effects upon the "in vitro" percutaneous absorption through rat skin of compounds with a wide range of lipophilicity values have been established (Borrás-Blasco et al., 2004).

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The objective of this study was to contribute new experimental data in order to analyze and compare the effects of two AHAs (glycolic and lactic acid) on the barrier function of the skin with another classic enhancer such as sodium lauryl sulphate. To this effect, we investigated the enhancing action of lactic and glycolic acid and also of sodium lauryl sulphate on the "in vitro" permeation through human epidermis of a series of compounds: 5-fluorouracil, 2-phenylethanol, 4-phenylbutanol and 5-phenylpentanol, with a broad range of lipophilicity values ($\log P_{\mathrm{oct}}$ from −0.95 to 2.89). On the other hand, the alpha-hydroxyacids and sodium lauryl sulphate have been tested for the "in vivo" determination in human volunteers of their irritative capacity, at the concentrations used in the diffusional experiments.

2. Material and methods

2.1. Compounds

Sodium lauryl sulphate (SLS), glycolic acid (GA) and lactic acid (LA) were purchased from Merck (Madrid, Spain) and had a stated purity of >98%. The permeants used in this study, i.e., 5-fluorouracil (5-FU), 2-phenylethanol (PHE), 4-phenylbutanol (PHB) and 5-phenylpentanol (PHP), were purchased from Sigma Chemical Co., Madrid, Spain, at >99% purity. The compounds were prepared as saturated solutions buffered to pH 6.2, with an excess of compound added to maintain saturation for the duration of the experiments.

2.2. In vitro diffusion studies

All diffusion studies were performed on Caucasian abdominal skin samples (females aged 30–40 years), obtained from cosmetic surgical corrections. Excess of fatty and connective tissues were removed. Epidermal membranes were prepared by a previously described heat-separation technique (Scott et al., 1986).

The epidermal membranes were placed in Franz-type diffusion cells with an effective area available for diffusion of 0.78 cm². The receiver compartment capacity was approximately 6 ml and the temperature was maintained at $37 \pm 1\,^{\circ}\mathrm{C}$ by immersion of the cells in a water bath. The receptor solution (buffered to pH 7.4) was added with polysorbate 80 at a clearly supramicellar concentration (1%, w/w) in order to provide a micellar reservoir and, consequently, sink conditions were completely fulfilled (Díez-Sales et al., 1991). Prior to the diffusional experiments, membranes were pretreated overnight with 2 ml of an aqueous solution of glycolic acid, lactic acid or SLS (1% and 5%, w/w, respectively). At zero time, a 2 ml aliquot of the saturated solution of the compound at pH 6.2 was applied to each donor compartment and covered with parafilm to prevent evaporation of solvents. Samples of 0.2 ml were taken from the receptor compartment over a 34-h period. The volume withdrawn was always replaced with an equal volume of fresh receptor solution. In order to test the integrity of skin samples (Hanafy et al., 2001), at the end of the experiments phenol red solution (0.5%, w/w) was added to the donor compartment (200 μl).

Quantification of the test compounds in the samples was done by HPLC using a Perkin-Elmer liquid chromatograph which includes a Binary LC Pump 250, a Rheodyne P/N 7125-047 model injector, a Perkin-Elmer, LC 90 UV detector set at 254 nm and an LCI-100 integrator. An analytical Novapak C-18 column (150/39 mm) was employed. The mobile phases were composed of mixtures of acetonitrile and phosphate buffer solution (pH 6.2) in variable proportions, depending on the lipophilicities of the tested solutes, and were delivered at a flow rate of 1 ml/min at room temperature. Previous to the HPLC analysis, no interference from the enhancers and from the skin components was verified. Calibration curves covering the entire range of concentrations assayed for the compounds were prepared in triplicate. The accuracy of the method was evaluated by calculating the relative error, which was always less than 9%, and precision was evaluated by calculating the variation coefficient, which was lower than 10% and is considered acceptable (Karnes and March, 1993). Other details of the method are described elsewhere (Díez-Sales et al., 1996; López et al., 2000; Borrás-Blasco et al., 2004).

The cumulative amount of drug permeated through the skin can be plotted as a function of time in accordance to the classic equation (Eq. (1)) representing the diffusional process:

$$Q_{(t)} = AKLC\left[D\frac{t}{L^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^2}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{L^2}\right)\right] \tag{1}$$

where $Q_{(t)}$ is the quantity which passes through the membrane and reaches the receptor solution at a given time t, A represents the actual diffusional surface area (0.78 cm²), K the partition coefficient of the permeant between the membrane and the donor vehicle, L the diffusional pathway, D the diffusion coefficient of the permeant in the membrane and C is the concentration (here solubility) of the permeant in the donor solution. The lag time ($t_{\mathrm{L}} = L^2/6D$) and permeability coefficient ($K_{\mathrm{p}} = KD/L$) expressions are obtained from Eq. (1). By substituting these expressions in Eq. (1) and by rearrangement, the following equation is obtained:

$$Q_{(t)} = AK_{\mathrm{p}}C\left[t - t_{\mathrm{L}} - \frac{12t_{\mathrm{L}}}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-n^2\pi^2 t}{6t_{\mathrm{L}}}\right)\right] \tag{2}$$

and when the steady state is reached, the representative equation of the process is a linear expression (Eq. (3)):

$$Q_{(t)} = AK_{\mathrm{p}}C[t - t_{\mathrm{L}}] \tag{3}$$

Permeability coefficients (K_{p}, cm h^{-1}) and lag times (t_{L}, h) through non-pretreated epidermis (control) and epidermis pretreated with the two AHAs (lactic and glycolic acids) and SLS were calculated from Eq. (3), which allows us to directly obtain these permeation parameters with an estimation of their precision. The number of data points on the straight line for all penetrants studied was seven (from 24 to 34 h) (Fig. 1). The fitting procedures were carried out by means of non-linear regression using the Sigma Plot 8.0 package (Jandel Scientific Corporation). An equal weighting scheme was applied.