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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 $\log P_{oct}$ −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 ($\log P_{oct} = 2.89$). 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 ($\log P_{oct}$ 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 cm^2. The receiver compartment capacity was approximately 6 ml and the temperature was maintained at 37 ± 1 °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:

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

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 cm^2), 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 ($t_L = L^2/6D$) and permeability coefficient ($K_p = KD/L$) expressions are obtained from Eq. (1). By substituting these expressions in Eq. (1) and by rearrangement, the following equation is obtained:

$$Q_{(t)} = AK_pC\left[t - t_L - \frac{12t_L}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-n^2\pi^2 t}{6t_L}\right)\right] \quad (2)$$

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

$$Q_{(t)} = AK_pC[t - t_L] \quad (3)$$

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.

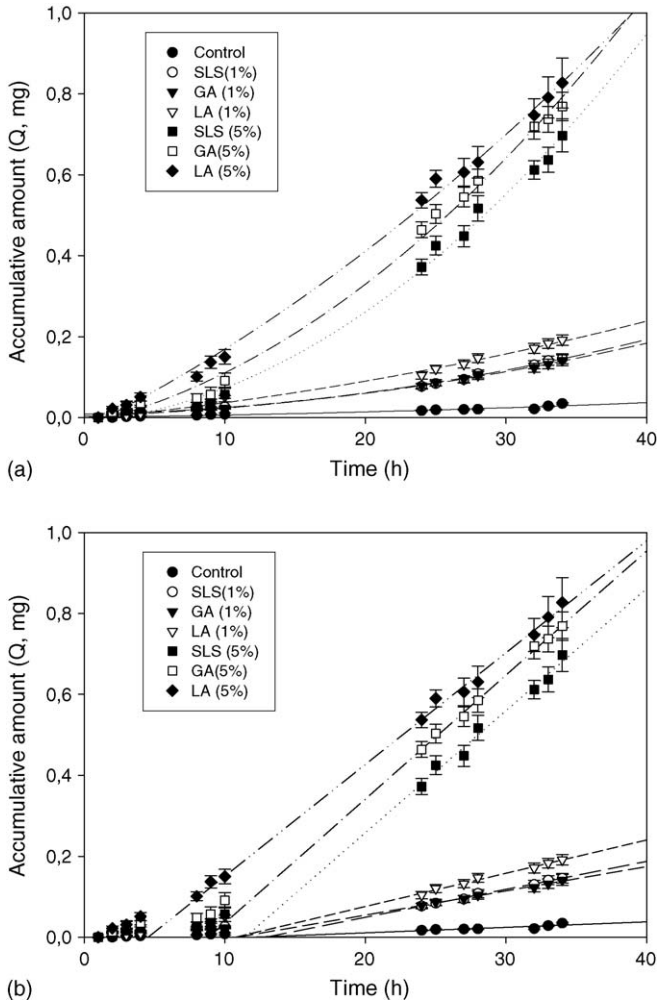

Fig. 1. Accumulated amounts of 5-FU for control and membrane pretreatment with the enhancers in the different experimental conditions (a) and data points on the straight line used for the K_p and t_L calculations (b) ($n = 4$, mean with error bars).

The enhancement ratio (ER) was calculated according the equation proposed by Williams and Barry (1991):

$$\mathrm{ER} = \frac{K_p \text{ with skin pretreatment}}{K_p \text{ without skin pretreatment}} \quad (4)$$

2.3. Skin irritation patch testing

Single application patch tests were conducted to determine whether the AHAs act as skin irritants in comparison with the effects of SLS. Thirty-two women aged 30–40 years, with phototypes II and III, were recruited and gave their informed consent to participation in the study.

Patch testing was performed with a semi-occluded Finn-Chamber®. A filter paper disc was placed in each chamber moistened with AHAs or SLS solutions. The tests were applied to healthy skin on the back, which was free of ointments and excessive sebum. The patch was kept in place for 24 h, after which the test site was observed for signs of irritation over the following 3 days. Skin irritation was assessed visually according to the following scale: (+?) doubtful reaction, (+) erythema with infiltration, (++) erythema, infiltration, papules, (+++) the same with the formation of vesicles, (++++) strong positive reaction with marked edema and confluent vesicles/bullae (De Groot et al., 1994).

2.4. Statistical analysis

The permeability coefficients and lag times were statistically evaluated by one- and two-tailed analysis of variance (ANOVA) after homogeneity was confirmed by Bartlett's test. Post-hoc comparisons of the means of individual groups were performed with Scheffe's test, with the level of statistical significance set to $P < 0.05$.

3. Results and discussion

The permeability coefficients (K_p) and lag times (t_L) for the compounds assayed through epidermis non-pretreated (control) and pretreated with GA, LA and SLS at the two concentrations considered (1% and 5%, w/w) are summarized in Table 1.

As could be expected, in accordance to the different partition–diffusion processes involved in skin absorption and the well-established skin permeability–lipophilicity relationships, the K_p values for non-pretreated (control) membranes increased with increasing lipophilicity of the penetrant, and an inverse

Table 1
Lipophilicity values ($\log P_{oct}$), permeability coefficients, K_p, and lag times, t_L, found for the tested compounds, 5-fluorouracil (5-FU), 2-phenylethanol (PHE), 4-phenylbutanol (PHB) and 5-phenylpentanol (PHP), for the different series of experiments carried out ($n = 4$, mean ± S.D.)

Experimental series	5-FU ($\log P_{oct} = -0.95$)[a]		PHE ($\log P_{oct} = 1.34$)[b]		PHB ($\log P_{oct} = 2.33$)[b]		PHP ($\log P_{oct} = 2.89$)[b]	
	K_p ($\times 10^3$ cm h^{-1})	t_L (h)	K_p ($\times 10^3$ cm h^{-1})	t_L (h)	K_p ($\times 10^3$ cm h^{-1})	t_L (h)	K_p ($\times 10^3$ cm h^{-1})	t_L (h)
Control	0.13 (0.03)	13.7 (0.7)	55 (4)	6.3 (1.3)	83 (7)	1.9 (0.6)	113 (14)	0.6 (0.1)
Epidermis pretreatment with 1% (w/w)								
SLS	0.63 (0.06)	12.9 (1.2)	120 (9)	3.5 (0.6)	101 (9)	1.9 (0.8)	110 (16)	0.8 (0.2)
GA	0.53 (0.05)	11.7 (1.2)	104 (10)	4.6 (1.5)	105 (11)	2.7 (0.5)	111 (1.5)	4.6 (1.5)
LA	0.72 (0.08)	11.5 (1.0)	98 (8)	5.6 (0.4)	103 (4)	1.7 (0.3)	84 (15)	1.5 (1.0)
Epidermis pretreatment with 5% (w/w)								
SLS	2.19 (0.15)	10.7 (1.0)	133 (7)	1.7 (0.7)	112 (7)	0.9 (0.3)	118 (17)	0.8 (0.3)
GA	2.33 (0.18)	8.7 (0.7)	122 (15)	3.5 (1.1)	103 (11)	2.2 (0.5)	103 (4)	1.4 (0.7)
LA	2.35 (0.19)	4.9 (0.5)	152 (22)	4.9 (0.5)	101 (12)	4.3 (1.1)	96 (11)	2.2 (1.0)

[a] Leo et al. (1971).
[b] López et al. (1998).

tendency was observed for the t_L values, which diminished with increasing penetrant lipophilicity.

There were significant differences ($P < 0.05$) between the permeability coefficients obtained through membrane without pretreatment (control) and with pretreatment with the two AHAs and SLS for compounds of $\log P_{oct} < 2.89$ (5-FU, 2-PHE and 4-PHB). In the case of the most lipophilic compound assayed (5-PHP), there were no statistically significant differences in permeability coefficient between the control and membrane pretreated with glycolic acid and SLS. However, membrane pretreatment with lactic acid yielded a statistically significant reduction in the K_p values for this compound.

The effect of the concentration used in membrane pretreatment was also dependent upon the lipophilicity of the penetrant. In fact, the K_p values calculated for 5-FU were always greater for the higher concentration (5%) than for the lower concentration (1%), for all the enhancers assayed. In the case of 2-phenylethanol, only pretreatment of the membrane with lactic acid solution at a concentration of 5% yielded a K_p value significantly greater than for a concentration of 1% (w/w) (Table 1).

In the case of 4-phenylbutanol and 5-phenylpentanol there were no statistically significant differences between the permeability coefficients obtained at the two membrane treatment concentrations for all of the enhancers studied.

Regarding the effect of membrane pretreatment upon penetrant t_L values, the latter tended to decrease (Table 1) for hydrophilic compounds and increase for the most lipophilic one (5-PHP), particularly when the membrane was pretreated with LA.

On the other hand, the enhancement ratios (ER) calculated from the permeability coefficients for all the permeants and conditions used in this study are reported in Fig. 2. Differences were recorded in enhancer behavior under the two conditions assayed, depending on the lipophilicity of the penetrants. To explain these differences, it is necessary to consider the different molecular mechanisms involved in diffusion through the stratum corneum of hydrophilic and lipophilic molecules (Blank et al., 1967), along with the principal mechanisms of action of percutaneous enhancers that have been described (Barry, 1987; Williams and Barry, 2004). As can be seen, for any particular skin pretreatment condition, the greatest enhancer effect corresponded to the lowest lipophilicity value of the penetrant (5-FU).

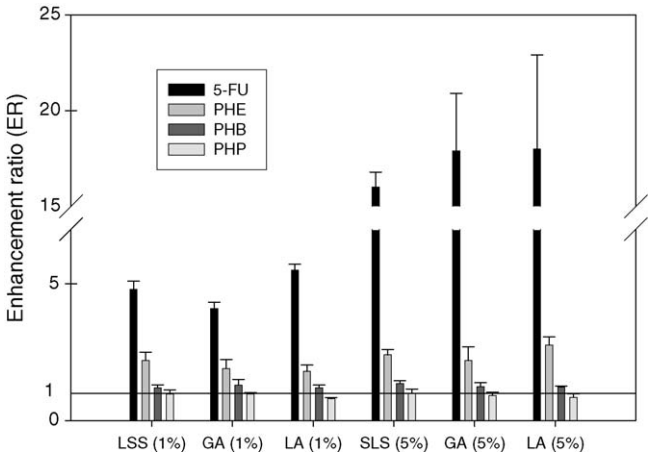

Fig. 2. The penetration-enhancing activity of sodium lauryl sulphate (SLS), glycolic acid (GA) and lactic acid (LA), for skin treatment at the two concentrations (1% and 5%) used in the experiments, expressed as enhancement ratios ($n = 4$, mean with error bars).

At first sight it could be considered that the results obtained for SLS-pretreated skin are similar to those previously obtained with this surfactant and other series of compounds in application to rat skin (Borrás-Blasco et al., 1997), and also with Azone in application to human skin (Díez-Sales et al., 1996). The effect of SLS as an enhancer could be attributed to the molecular insertion of this surfactant into lipid bilayers of the stratum corneum (Bouwstra et al., 1989), as occurs with Azone and other surfactants such as Span 20 (López et al., 2000). In fact, SLS possesses the same C12 alkyl chain as Azone and Span 20. The local disorders induced by such insertion could allow an increase in drug permeation mainly via the intercellular route (Ribaud et al., 1994). However, the marked differences in the permeability coefficients observed in relation to the lipophilicity of the penetrants cannot be explained by this mechanism alone. As previously described for Azone (Díez-Sales et al., 1996), it seems that SLS could also affect the polarity of the stratum corneum because hydrophobic interaction of SLS alkyl chain with the skin structure leaves the end sulphate group of the surfactant exposed, creating additional sites in the membrane which could permit an increase in the skin hydration—this latter effect allowing polar molecules to partition across the barrier more easily. This effect of SLS may also be related to the extraction of various components from the intercellular lipid matrix of the stratum corneum (Lodén, 1990; Ridout et al., 1991), particularly when the surfactant is used at concentrations above the critical micelle concentration in aqueous vehicle (>0.24%). However, the disruption of the stratum corneum and the hydration of the stratum corneum and viable epidermis could cancel each other out in the case of the most lipophilic compound (PHP).

Regarding the behavior as enhancers of the two AHAs used in the study, they globally could be considered qualitatively similar to SLS. Since they lack the typical structure of surfactants, (i.e., a lipophilic chain and a polar group), the effects of AHAs cannot be attributed to any local disorder induced by insertion between the intercellular lipids within the stratum corneum. Probably the effects of AHAs are attributable to their hygroscopic properties and to skin penetration capability itself (Kraeling and Bronaugh, 1997). In general, increased tissue hydration appears to increase transdermal delivery of both hydrophilic and lipophilic permeants (Williams and Barry, 2004), but the results of the present work and other results from Bucks and Maibach (1999), such a generalization does not appear to be realistic. At first it is important to note that for 5-fluorouracil, the enhancement ratios obtained in the present work for membrane pretreated with 1% lactic acid were slightly higher than for membrane pretreated with glycolic acid and SLS solution at the same concentration (1%) (Fig. 2). When the concentration of pretreatment solution used was high (5%), the enhancement ratios obtained for both AHAs were significantly higher than for skin pretreated with SLS solution (Fig. 2). It seems that AHAs exert a skin hydration effect (Williams and Barry, 2004), which produces an increase

in partition into the skin of hydrophilic or low lipophilic compounds: $\log P_{oct} < 2.89$ (5-FU, PHE and PHB) and promotes their permeation capacity through the skin. Nevertheless, for the most lipophilic compound (PHP: $\log P_{oct} = 2.89$), SLS and glycolic acid exerted no effect, while for skin pretreated with lactic acid (1% and 5%), partition into the "hydrated" stratum corneum was made difficult—with a reduction in its permeation capacity through the skin (ER < 1). This effect seems to be confirmed by the increase in the lag time value because of the inverse dependence between diffusional parameter (D) and lag time ($t_L = L^2/6D$). The superior potency of lactic acid as a hydrant possibly could be a result of increased retention within the stratum corneum—as can be deduced from the data presented by Kraeling and Bronaugh (1997).

It seems that the results obtained for lipophilic compounds in this work and explained on the basis of an "hydration skin" effect by the AHAs, are in contradiction to the in vivo-occlusion data from Bucks and Maibach (1999). Their results showed that a trend of occlusion-induced absorption enhancement with increasing penetrant lipophilicity is apparent. The authors postulate that increasing of hydrophilic character of the stratum corneum leads, in turn, to a reduction in the stratum corneum-viable epidermis partition coefficient of the penetrant (because the two tissue phases now appear more similar); this effect should facilitate the kinetics of transfer of penetrant to the viable epidermis and the relative effect on this rate should be greater as the lipophilicity of the absorbing molecule increases. Certainly there are great differences in the respective in vivo and in vitro experimental techniques. In the in vitro present work an infinite dose of penetrants is used and perhaps the reduction of stratum corneum corneocyte cohesion by AHAs that has been suggested (Kraeling and Bronaugh, 1999) could also take a contribution on the global effect of these enhancers which favors hydrophilic permeants.

Ideally, percutaneous penetration enhancers should be pharmacologically inert and have an immediate but reversible effect on the stratum corneum. It is well known that SLS binds extensively to intracellular keratin, which explains its irritant skin effects (Leveque et al., 1993). For comparative purposes, and with the aim of selecting the best candidate for use as a percutaneous penetration enhancer, the reaction indexes obtained from primary skin irritation testing in the range of concentrations assayed are shown in Table 2. As can be seen, at the concentration used in the experiments (1%), SLS acts as a skin irritant in about 50% of the cases. This value is greater than the percentages obtained for both AHAs at the two concentrations assayed (1% and 5%, w/w). Moreover, on comparing the reaction indexes obtained for the two AHAs, it can be seen that for both concentrations assayed, LA produces irritant effects in a lower percentage of cases than GA.

Table 2
Results obtained from primary skin irritation testing (24 h) carried out with glycolic acid (GA), lactic acid (LA) and sodium lauryl sulphate (SLS) ($n = 32$)

Compounds	Reaction index				Total	%
	+?	+	++	+++	Clearly positive	
GA (1%)	0	2	0	0	2	6.3
GA (5%)	4	6	0	0	6	18.8
LA (1%)	0	1	0	0	1	3.1
LA (5%)	2	2	0	0	2	6.3
SLS (1%)	1	14	2	0	16	50.0

(+?) Doubtful reaction, (+) erythema with infiltration, (++) erythema, infiltration, papules and (+++) the same with the formation of vesicles (De Groot et al., 1994).

In conclusion, on taking into account that the highest percutaneous penetration enhancer potency for 5-fluorouracil corresponded to lactic acid while the lowest irritant capacity corresponded to this AHA, we could propose lactic acid as a percutaneous penetration enhancer for hydrophilic molecules of interest for transdermal administration.

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