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The influence of skin moisturizers on drug penetration in vivo

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Summary

The flexor forearms of volunteers were treated with 10% skin moisturizing solutions – *N*-hydroxyethyllactamide (OH-Lac), sodium lactate (Na-Lac), sodium pyroglutaminate (Na-PCA), sorbitol and urea – with 10% of the hydrophilic penetration enhancer propylene glycol (P-glyc) and water, respectively. Significant differences in the biological activity of benzyl nicotinate were observed after its application as petrolatum ointment at different concentrations on the pretreated skin. The resulting concentration-response curves, based on the latency time to induce an erythema, were shifted on the *x*-axis to higher concentrations with all moisturizers except urea, demonstrating significant penetration deceleration. The concentration in the ointments required to be raised by a factor of 2.2 (sorbitol) to 6.7 (Na-Lac) in order to elicit the same response as in the case of pretreatment with water. Propylene glycol enhanced penetration, as expected from a bioavailability factor of 2.59. It may be hypothesized that the moisturizers compete for water of the keratin in the corneocytes and of the polar regions in the lipid bilayers, and thus even decrease the fluidity of the lipids. As a consequence, the resistance of both the intra- and intercellular penetration pathways is increased.

Introduction

Topical penetration enhancers of the hydrophilic type, such as DMSO, the pyrrolidones, dimethylacetamide, dimethylformamide, urea, propylene glycol and other glycols, increase the extent of hydration of the stratum corneum (Tagami et al., 1980; Stüttgen, 1984; Wohlrab, 1984; Barry, 1987; Walters, 1989). They may act similarly to occlusion, inducing a greater degree of aqueous solvation of the polar regions of the intercellular lipid bilayers together with its fluidization and of the keratin within the corneocytes (Barry, 1987; Walters, 1989). The naturally occurring moisturizing factor (NMF), containing especially amino acids, pyrrolidone-5-carboxylic acid and lactic acid as sodium salts and urea, is responsible for the water-binding capacity of the stratum corneum (Smolle et al., 1986). Its influence on the permeability of the stratum corneum is incompletely understood.

All these compounds are more or less hygroscopic (Hüttinger, 1978; Gloor, 1982; Hackemüller, 1988; Sugibayashi et al., 1988), induce increased water uptake of the skin (Jacobi, 1967; Middleton and Marese, 1978; Tagami et al., 1980; Clar, 1981; Wienert et al., 1981; Gloor, 1982;

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Klaschka, 1982; Stüttgen, 1984; Smolle et al., 1986; Hackemüller, 1988); and may act as moisturizers. Some are used for this purpose in therapeutics and cosmetics (Middleton and Marese, 1978; Franks and Lieb, 1980; Clar, 1981; Gloor, 1982).

The intention of this investigation was to determine the influence of some NMF substances – sodium lactate (Na-Lac), sodium pyroglutaminate (Na-PCA), urea – of cosmetic moisturizers and related compounds – sorbitol, *N*-hydroxyethyllactamide (OH-Lac) – and of a well-known hydrophylic penetration enhancer – propylene glycol (P-glyc) – on the penetration of a model drug through the skin. Thus, it was considered to be possible to deduce whether hygroscopicity and water binding ability of a substance are important factors for penetration enhancement.

Materials and Methods

Moisturizers and related compounds

Propylene glycol (Ph. Eur.; P-glyc), *N*-hydroxyethyllactamide (OH-Lac; Boehringer-Ingelheim, Ingelheim, F.R.G.; water content, 30%), sodium lactate (Ph.Eur.; Na-Lac; water content, 50%), sodium pyroglutaminate (sodium salt of DL-2-pyrrolidone-5-carboxylic acid; Na-PCA; Ajidew(R), Ajinomoto, Tokyo, Japan; water content, 50%), sorbitol (Ph.Eur.) and urea (Riedel-de-Häen) were used.

These compounds were used as a 10% solution, pH 6.1–6.3 (adjusted with HCl in the case of OH-Lac), referred to the pure substance.

Ointments

Solution-type ointments of benzyl nicotinate (Merck, Darmstadt, F.R.G.) in soft white petrolatum of different concentrations in the range of 10^{-3}% w/v, 0.313–3% (w/v). The concentrations are duplicated from one to the next (except the last one).

Comparative activity test

The volunteers, 13–36 years old, were informed about the test and gave written consent to participate. They submerged one forearm in a moisturizer solution, the other in deionized water for 20 min. They dried their wet arms on air for another 30 min. Benzyl nicotinate ointments at eight different concentrations and petrolatum as blank were applied on circular areas of 15 mm diameter on the inner side of each forearm. The amount of ointment was adjusted to 5 μl with a metered applicator (Lippold and Reimann, 1989) and spread out, resulting in a layer of ointment of 28 μm. The different concentrations were randomly distributed over the areas. A given concentration was applied at identical positions on the right and left arms to check for positional effects (intraindividual variations). The time between application and the occurrence of visible redness (erythema), i.e., the latency time t, was measured via the double blind method.

Data treatment

The reciprocal of the latency time to redness, $1/t$, provides a measure of the rate and extent of penetration of benzylnicotinate, as do temperature increase, intensity of red coloration, duration of the effects and others (intensity-duration parameters) (Lippold and Teubner, 1981a, b). All these parameters increase with increase in both penetration rate and amount absorbed, since more drug will be present at the receptors at a given time. The value $1/t$ reflects the penetration rate alone, if the same amount of drug is absorbed. By plotting $1/t$ vs the logarithm of the concentration, dose-response curves (concentration-effect curves) are obtained (Lippold and Teubner, 1981a; Lippold and Reimann, 1989). Using the pretreatment with water as standard the concentration-response curves obtained after pretreatment with a moisturizer (test) are shifted parallel to the x-axis to lower concentrations in the case of penetration enhancement and to higher concentrations for reduction of penetration. The horizontal distance from the standard to the test curve at a fixed response level (medium effect) gives log f, f being the relative bioavailability factor. $1/f$ describes the extent of the increase/decrease in concentration of the ointment required after pretreatment of the skin with moisturizer to yield the same response as in the case of the standard application with water pretreatment. Assuming an equally large extent of penetration (Lippold and Reimann,

1989), f provides a direct measure of the relative penetration rate constant, related to the standard (Lippold and Teubner, 1981a; Lippold and Schneemann, 1984; Lippold and Reimann, 1989). For example, a bioavailability factor of 2 after pretreatment with moisturizer signifies a 2-fold higher penetration rate constant in comparison to pretreatment with water (standard).

In some instances, the individual concentration-response curves show deviations from linear and/or parallel behavior in the steep region and the 95% confidence limits of the $1/t$ medians for each concentration increase with concentration. As described before (Lippold and Reimann, 1989), the former may be due to emptying of drug from the ointment reservoir at low concentrations, the latter to lack of differentiation in the measurement of t at high concentrations (range of minutes) and formation of the quotient $1/t$ which increases the variability. Therefore, transformation of the $1/t$ values, as is often performed with time data (Lienert, 1962; Dolby, 1963; Finney, 1964; Holford and Steiner, 1981; Lippold and Schneemann, 1984; Lippold and Reimann, 1989), is carried out. The use of $1/t^{1/2}$ seemed appropriate and was therefore employed, similar results being obtained with a logarithmic transformation. Fig. 1 shows

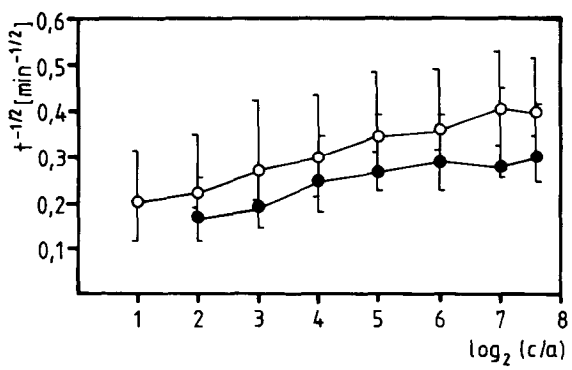

Fig. 1. Concentration-response curve with the response parameter $t^{-1/2}$ of benzyl nicotinate in soft white petrolatum; pretreatment with Na-Lac (●) and water (○), respectively; medians ($n = 10$ volunteers) with 95% confidence limits; $a = 0.015625$ is a transformation factor to give whole numbers for the logarithms of c/a to the base 2.

the concentration-response curve for $t^{-1/2}$ after pretreatment with water and Na-Lac of 10 volunteers, and the medians with 95% confidence limits. However, these median curves were not used for evaluation of f due to the high interindividual variations (different sensitivity to benzyl nicotinate). Nevertheless, it should be noted that pretreatment with moisturizer in almost every case produced the same effect: Na-Lac resulted in longer t values in 149 cases out of 150 (most potent), sorbitol in 153 cases out of 157 (weakest activity).

According to bioavailability studies, the relative bioavailability factor f should be determined from the individual pairs of dose-response curves via Eqn. 1 (Finney, 1964; Cavalli-Sforza, 1974; Steinijans and Diletti, 1983; Lippold and Schneemann, 1984; Lippold and Reimann, 1989) in order to exclude inter-individual variations:

$$\log f = \bar{x}_{ST} - \bar{x}_T - \frac{\overline{W}_{ST} - \overline{W}_T}{\bar{b}} \qquad (1)$$

where $\bar{x}_{ST}$, and $\bar{x}_T$ denote the means of the log ointment concentration used on the skin, pretreated with water and moisturizer, respectively; $\overline{W}_{ST}$ and $\overline{W}_T$ are the means of the response (transformed latency time) after application of the ointments on the skin, pretreated with water and moisturizer, respectively; and $\bar{b}$ is the mean slope for the two concentration-response curves, supposing its linear and parallel nature (Diem and Lentner, 1975).

In agreement with bioavailability studies (Steinijans and Diletti, 1983), first the mean of the individual log f values with log confidence limits and then the mean f values with unsymmetrical confidence limits for pretreatment with moisturizer are calculated. The test on significant differences between pretreatment with water ($f = 1$) and moisturizer, respectively, is carried out with 95% confidence limits (bilateral). A significant difference exists if the confidence limits of the f values of the tests do not include the bioavailability factor 1 of the standard. A relative bioavailability factor higher than 1 signifies enhancement, and smaller than 1 deceleration of penetration.

Results and Discussion

Table 1 lists the latency times t for different concentrations of benzyl nicotinate in petrolatum ointment after pretreatment with various moisturizers in comparison to water (medians with 95% confidence limits). Fig. 2 shows the influence of the moisturizers on the relative bioavailability factor f. Surprisingly, pretreatment with Na-Lac, Na-PCA, OH-Lac and sorbitol decreased f significantly to 0.15, 0.36, 0.36 and 0.45, respectively; $f = 1$ was not included in the 95% confidence limits. Urea remains without significant influence ($f = 0.93$; confidence limits include $f = 1$); propylene glycol significantly enhances f as expected from the factor 2.59. In other words, the applied concentration, for example, must be 6.7-times higher in the case of Na-Lac pretreatment and requires to be reduced to nearly one third in the case of propylene glycol to obtain the same latency time as with water pretreatment.

Propylene glycol is well known as a penetration enhancer (Barry, 1987; Walters; 1989). However, its precise mode of action remains unclear. It may act as a cosolvent in the skin, increase the partition coefficient for stratum corneum/vehicle and hydrate α-keratin and fluidize lipid bilayers via their hydration. In this study, the observed penetration enhancement with propylene glycol demonstrates the validity of the experimental set-up, since its expected effect is clearly evident, taking the pretreatment with Na-Lac as a stan-

TABLE 1

Latency time t (min) for benzyl nicotinate in petrolatum ointment after pretreatment with different moisturizers (test) and water (standard), medians with 95% confidence limits, n = 10 (n.r., no reaction)

Pretreatment (test and standard)	Concentration (g/100 ml)							
	0.0313	0.0625	0.125	0.250	0.50	1.0	2.0	3.0
P-glyc	27.10	16.37	11.06	9.08	6.33	5.75	5.23	5.38
	(17.58/n.r.)	(13.42/27.78)	(9.15/15.77)	(7.10/11.19)	(4.83/9.80)	(4.16/7.93)	(4.01/8.33)	(4.07/6.80)
Water	30.30	23.75	15.02	12.77	8.35	7.32	7.01	6.68
	(27.47/n.r.)	(16.56/29.41)	(12.18/18.42)	7.32/13.81)	(7.33/11.61)	(6.85/11.60)	(5.30/10.32)	(4.38/9.00)
OH-Lac	45.66	26.04	19.38	13.26	10.98	8.71	9.33	9.06
	(35.84/n./r.)	(21.14/50.76)	(10.47/31.15)	(10.40/21.88)	(9.20/14.23)	(6.32/17.34)	(7.05/12.80)	(5.18/12.82)
Water	24.57	18.48	13.35	12.09	9.00	8.44	7.77	7.63
	(16.42/43.10)	(10.83/33.00)	(9.10/21.51)	(8.67/14.54)	(4.63/12.63)	(4.63/10.63)	(4.63/8.95)	(3.78/9.30)
Na-Lac	n.r.	38.24	27.78	16.37	14.12	11.95	12.97	11.27
	(n.r./n.r.)	(15.20/74.63)	14.07/47.17)	(8.38/30.86)	(6.54/19.80)	(6.50/19.53)	(4.95/15.46)	(5.77/16.95)
Water	31.25	20.12	13.62	11.26	8.41	7.84	6.15	6.45
	(10.10/74.07)	(8.10/27.40)	(5.53/23.10)	(5.28/21.55)	(4.23/10.43)	(4.13/10.02)	(3.52/9.45)	(3.77/8.38)
Na-PCA	50.25	28.82	15.13	14.95	8.76	8.10	6.14	6.55
	(28.82/n.r.)	(21.79/44.44)	11.88/21.05)	(10.33/17.48)	(5.31/12.33)	(5.27/9.70)	4.10/8.63)	(4.58/10.48)
Water	25.51	13.77	10.81	8.80	7.47	6.28	4.57	5.35
	(19.27/34.25)	(8.57/23.47)	(5.92/14.27)	(6.87/10.76)	(4.70/9.10)	(4.23/7.70)	(3.73/7.00)	(3.50/7.90)
Sorbitol	38.46	24.41	22.08	12.76	8.66	9.02	6.65	5.96
	(21.43/n.r.)	(15.67/32.68)	(13.28/26.88)	(6.87/19.72)	(7.17/13.61)	6.57/15.67)	(5.35/11.92)	(5.40/10.93)
Water	26.46	17.58	15.29	9.45	7.91	7.26	5.42	6.27
	(16.84/65.36)	(13.12/21.23)	(11.15/16.18)	(6.35/13.79)	(4.58/12.05)	(4.40/12.05)	(4.17/9.97)	(4.48/8.18)
Urea	29.33	19.92	15.58	12.69	9.84	9.41	7.84	6.52
	(24.39/n.r.)	(11.78/36.23)	(5.85/21.14)	(5.32/19.88)	(5.78/22.03)	(4.75/14.97)	4.80/11.75)	(5.37/9.48)
Water	35.34	22.13	17.21	12.79	10.92	8.24	6.56	7.08
	(22.12/n.r.)	(11.03/39.22)	(8.67/23.75)	(9.13/16.61)	(5.93/16.92)	(4.71/13.99)	(5.80/11.43)	(5.18/10.61)

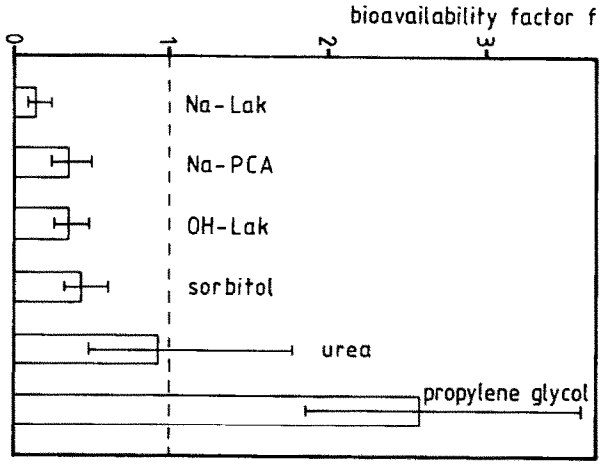

Fig. 2. Influence of pretreatment with moisturizers, related substances and propylene glycol on the bioavailability of benzyl nicotinate from soft white petrolatum; pretreatment with water (standard): $f = 1$; f as means ($n = 10$ volunteers) with 95% confidence limits.

dard, the f value of propylene glycol amounts to 15, thus making the differing strengths of the effects of the various moisturizers, distinctly evident.

The finding that urea had no significant influence may be due to the fact that it crystallized visibly on the skin after drying which followed the pretreatment, thus hindering the observation of redness. Other investigators observed only slight effects on coadministration of hexyl nicotinate with urea (Beastall et al., 1986).

Studies with other vehicles (o/w and w/o emulsions, polyethylene glycol ointment, methylcellulose gel) and Na-Lac as moisturizer demonstrated an influence of the vehicle when Na-Lac was tested in comparison with water pretreatment. However, significant penetration reduction was observed in all cases ($f = 0.25$, 0.31, 0.45 and 0.69, respectively). A study with the more hydrophilic drug methyl nicotinate (partition coefficient in octanol/ water = 1.24, but 211 for benzyl nicotinate), applied in a liquid paraffin-polyethylene gel, showed an almost equal extent of penetration reduction to that with Na-Lak. The extremely lipophilic betamethasone-17-benzoate (partition coefficient about 12440) also penetrates significantly more slowly, if applied in a w/o emulsion together with moisturizers (Na-Lac, Na-PCA, sorbitol and others) as compared to the same vehicle without additives (Hackemüller, 1988).

Thus, penetration reduction by these moisturizers appears to be a general phenomenon. They may act in different ways on the process of penetration. During treatment of the skin with aqueous solutions of moisturizers, they diffuse into the stratum corneum together with water and increase the overall water content (see Introduction). However, they supposedly compete with the water in the corneocytes and in the polar regions of the intervening lipid bilayers. Thus, the hydration of keratin and lipid polar head groups is reduced. Diminished hydration of the bilayers was proved to decrease the fluidity or melting range of such systems (Hauser, 1975; Blume, 1985; Nimitz, 1986;

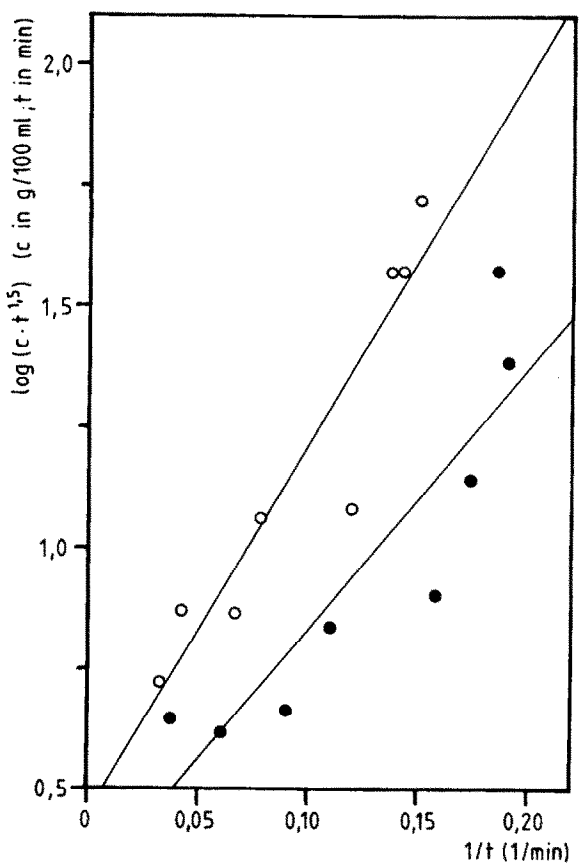

Fig. 3. Plot of $\log(c \cdot t^{1.5})$ vs $1/t$ according to Beastall et al. (1986), for pretreatment with P-glyc (●), and water (○) as standard; medians, $n = 10$; regression line.

Hübner and Blume , 1987) and was demonstrated even with saccharose in vitro (Cevc, 1988). The effect of the moisturizers may also be seen in connection with the structure of water. At least Na-Lac and sorbitol increase the structuring of water as may be demonstrated by the decreased diffusion coefficients and solubilities of drugs in such solutions. Therefore, the moisturizers absorbed by the stratum corneum could also decrease the partition coefficients of nicotinate esters between the skin and ointment, thus reducing its rate of penetration. To provide information on the mechanism of action of the moisturizers, log $c \cdot t^{1.5}$ was plotted vs $1/t$ (c = concentration of benzyl nicotinate in ointments), for both the test and respective standard. An alteration in the slope of the linear standard curve (water pretreatment) without inducing large differences in the intercept would indicate a change in diffusional resistance after pretreatment with the moisturizer (Beastall et al. 1986; Albery and Hadgraft, 1979). This can be clearly demonstrated for P-glyc and Na-Lac (Figs. 3 and 4), with an increasing slope for Na-Lac and a decreasing slope for P-glyc, the most effective compounds studied in this investigation. In the case of the less effective OH-Lac, Na-PCA and sorbitol, the slopes appear to decrease, the intercepts being a little greater. The latter could indicate a change in partition coefficient of the drug (Beastall et al., 1986; Albery and Hadgraft, 1979). However, the less pronounced activity of these substances and the scatter in the data do not allow a decision on the mechanism of action involved in this case.

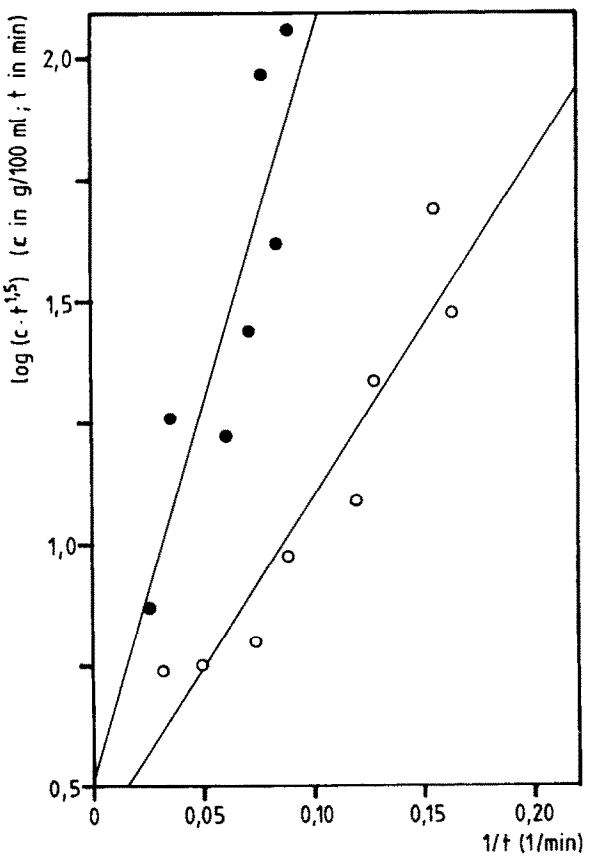

Fig. 4. Plot of $\log(c \cdot t^{1.5})$ vs $1/t$ according to Beastall et al. (1986) for pretreatment with Na-Lac (●), and water (○) as standard; medians, $n = 10$; regression line.

Because the studied moisturizers Na-Lac as well as Na-PCA, OH-Lac and sorbitol reduce the penetration of both lipophilic and hydrophilic drugs, it may be hypothesized that they influence keratin and the lipid bilayers (intra- and intercellular pathway).

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