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The influence of ethanol on permeation behaviour of the porous pathway in the stratum corneum

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Abstract

Relationship between in vitro percutaneous penetration of baclofen, a model zwitterion, and ethanol concentration in the vehicle was studied. In comparison with water, 95% ethanol increases the permeability coefficient of baclofen by a factor of 10, which suggests rapid and maximal pore formation in the stratum corneum. A slow time-dependent increase of penetration rate was observed for lower ethanol concentrations. For ethanol concentrations in the range 0–70%, solubility of baclofen in the vehicle is a dominant factor influencing the penetration rate. The reasons for an unexpectedly small effect of 70% ethanol on the process of pore formation in the stratum corneum are discussed.

Passive transport of ions across the skin was neglected in the past and even nowadays, regardless of some new evidence, it is not very well recognized. Despite the scarcity of data, a new physical model of skin was developed a few years ago (Berner and Cooper, 1987; Ghanem et al., 1987). According to this model, the stratum corneum is composed of two parallel pathways: the lipoidal pathway and hydrophilic porous pathway. The pores are aqueous regions in the stratum corneum, but it is still unclear whether they are located intra- or intercellularly and their characteristics are also unknown. The porous route is the most likely pathway for penetration of ionized drugs across the stratum corneum.

Good models for studying percutaneous penetration of ions are zwitterions since they are ionized in the whole pH range, including isoelectric point. Thus percutaneous absorption of amino acids, histidine, lysine and aspartic acid, as well as baclofen, a muscle relaxant, also amino acid in structure, was studied (Sznitowska et al., 1993; Sznitowska and Berner, 1995; Sznitowska and Janicki, 1995). In vitro passive transport of amino acids was observed with permeability coefficients $1.1–4.7 \times 10^{-8}$ cm/s and the porous pathway was indicated as a site of this transport in the stratum corneum.

In order to study the nature of the porous hydrophilic route, the effect of ethanol on percutaneous penetration of zwitterions was investigated. In previous studies 30% ethanol did not cause any enhancement of the penetration rate of histidine and lysine; moreover the flux of the

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latter was even decreased (Sznitowska et al., 1995). It was suggested that the lower solubility of baclofen in 30% ethanol than in water was responsible for the failure of ethanol to enhance the flux of these amino acids and that was consistent with the diffusion of ions through the pores in the stratum corneum.

In the present work, the influence of ethanol-water systems on in vitro percutaneous penetration of baclofen was studied. Baclofen was kindly given by Research Biochemicals Incorporated (Natick, MA, USA). The human skin was mounted in two-chamber flow-through teflon diffusion cells (Crown Glass Company, Somerville, NJ, USA) with a penetration area of 0.64 cm^2. The receiver was 0.9% sodium chloride solution recirculated beneath the dermal side of the skin. Saturated solutions of baclofen in ethanol-water systems (at 37°C), separated from the undissolved particles by filtration, were applied on the skin as donor phases. Every 12 h, up to 60 h, the receiver solution was entirely removed and replaced with fresh saline. Every 24 h the donor solution was also exchanged.

Ethanol 95% (v/v) produced by Polmos (Starogard Gd., Poland) was used to prepare the ethanol-water systems. The resulting donor solutions contained 0, 30, 70 or 95% (v/v) ethanol. Fig. 1 illustrates the influence of ethanol concentration in the mixture on baclofen solubility. Solubility in 30% ethanol is equal to that in 70% ethanol, and only slightly lower than in water.

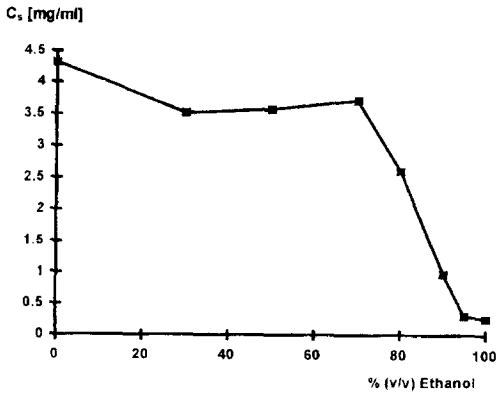

Fig. 1. Solubility of baclofen as a function of the ethanol concentration in ethanol-water mixtures.

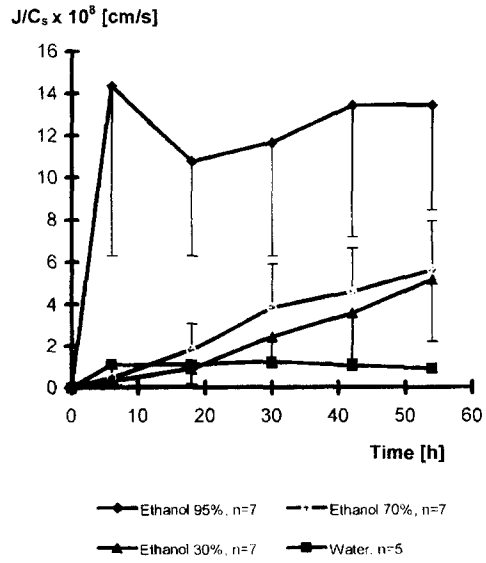

Fig. 2. Normalized penetration rate of baclofen through human skin in vitro from the ethanol-water mixtures containing various concentrations of ethanol. All data represent the mean ± S.D.

For the systems containing more than 70% ethanol it drops sharply and it is more than ten times lower in 95% ethanol than in water.

HPLC analysis was performed in order to measure the concentration of baclofen in the sampled receiver fluid using a Merck-Hitachi (Darmstadt, Germany) HPLC apparatus and LiChrospher RP-18 column (Merck). The mobile phase consisted of acetonitrile/methanol/0.02 M potassium dihydrogen orthophosphate (5:15:80, by wt.); pH 3.5 was adjusted with phosphoric acid. Baclofen was detected at 220 nm.

The results of the permeation experiment are presented in Fig. 2, where the penetration rate (J) was normalized with respect to the concentration of baclofen in the donor phase (C_s). The fluxes of baclofen from pure aqueous or ethanolic systems were 0.18 ± 0.05 and 0.14 ± 0.07 $\mu g/cm^2$ per h, respectively. The fluxes were equal, although diffusion through a porous structure must depend on the permeant solubility in the solvent, which actually fills the pores and decreased flux is rather expected for the ethanolic solution due to the decreased solubility of baclofen in 95% ethanol.

The calculated permeability coefficients K_p are 1.1×10^{-8} cm/s for the aqueous vehicle and 1.0×10^{-7} cm/s for the ethanolic vehicle and the difference indicates that ethanol enhances permeation of baclofen. In both cases T_{lag} was short, less than 6 h, as opposed to 20–30 h observed in the previous experiments when penetration of other amino acids from aqueous solutions was investigated (Sznitowska et al., 1993).

Regarding K_p values no other sorption promoter caused such an increase in permeation and the effect observed for 95% ethanol was maximal (Sznitowska and Janicki, 1995). Thus, 95% ethanol causes rapid and maximal pore formation in the stratum corneum. Although it has not been well documented yet, this effect is attributed to lipid extraction or/and conformational alterations within the protein domain in the presence of high concentrations of ethanol (Kurihara-Bergstrom et al., 1990).

Permeation of baclofen from 30% and 70% ethanol did not reach a steady state and permeation rate was constantly increasing. A period of 12–18 h was required to equal the penetration rate to that from the aqueous vehicle, while at 54 h it was four to five times greater for the ethanol-water systems than from water alone. Although mean penetration rates from 70% ethanol were greater than from 30% the difference was small. The same solubility of baclofen in both systems, 30% and 70% ethanol, is probably the reason for the similar penetration profiles.

The solubility in 30% or 70% ethanol is almost the same as in water; thus, similar penetration profiles may be expected from all three vehicles, but this is not the case. Higher penetration rates for ethanolic solutions despite the same solubility suggest that the number of pores, their volume or tortuosity is changed. This may be the effect of extraction of some lipids by ethanol. The increase of the porosity of the stratum corneum as a result of ethanol activity in concentrations as low as 30% has been already considered by other authors (Ghanem et al., 1992). Our results indicate that this effect is rapid and maximal when 95% ethanol is used as a vehicle, while for lower concentrations of ethanol it is continuous and results in a constantly increasing penetration rate of baclofen.

It is surprising that almost the same penetration profiles were obtained in the presence of 30% and 70% ethanol while other studies reported in the literature show significant differences. Ghanem et al. (1992) concluded that 70% ethanol, as opposed to 30% ethanol, acted almost as efficiently as 100% ethanol in the new pore formation process. It is worth noting that the K_p values for mannitol and tetraethylammonium bromide in the presence of high ethanol concentration were nearly the same as obtained in the present study although the other authors used hairless mouse skin. It has been also reported that the maximum flux of ethanol across human skin in vitro occurred when its concentration was 50–70% in a mixture with water (Berner et al., 1989). Kurihara-Bergstrom et al. (1990) found that 63% ethanol was the most effective in promoting transdermal penetration of the salicylate ion: K_p was 7.03×10^{-7} cm/s, while for 95% and 100% ethanol it was half of this value. In the present study the K_p values were in the same range; however, $K_p = 10 \times 10^{-7}$ cm/s was obtained for 95% ethanol while maximum penetration rate (at 54 h) from 70% ethanol was two times smaller than from 95% ethanol when the fluxes were normalized. It is difficult to explain such a small effect of 70% ethanol on the pore formation — maybe diffusion of water from the receiver compartment causes a serious dilution of ethanol in the stratum corneum, thus its activity is lower and not sufficient to promote drastic changes in the structures of the stratum corneum. Differences in solubilities of the permeants in ethanol/water mixtures and a zwitterionic character of baclofen may also be reasons for different results.

To exclude osmotic effects and changes in skin hydration the same solvent was used as a donor and receiver phase (symmetric model). When 95% ethanol was used on both sides of the skin no flux of baclofen was observed from the donor to the receiver phase. It was the result of a denaturing effect of 95% ethanol on the tissue or a complete dehydration of the skin. The results obtained for 30% and 70% ethanol are presented in Fig. 3 and compared with those obtained when saline served as the receiver phase.

The penetration profiles are similar when symmetric or asymmetric models of the experiment are considered; the difference, however, is that, in the symmetric model, steady-state conditions are achieved after 30 h. This may indicate that a maximal pore formation occurred. For 30% ethanol the steady-state flux of baclofen in the symmetric model is equal to the penetration rate at 60 h for the asymmetric model. It is surprising, however, that 70% ethanol did not cause significantly higher penetration of baclofen, when backflow of water is eliminated. In contrast, the penetration rate was even smaller than from 30% ethanol. One of the possible explanations is that, in this range of ethanol concentrations, differences in the ability of ethanol to produce pores are small and permeant solubility in the vehicle is the most important factor. The other explanation is more probable — not only 95% ethanol but also 70% ethanol, when used as the receiver fluid, causes serious dehydration of the tissue or even denaturation of proteins on the dermal side of the skin which reduces ionic transport to the receiver fluid.

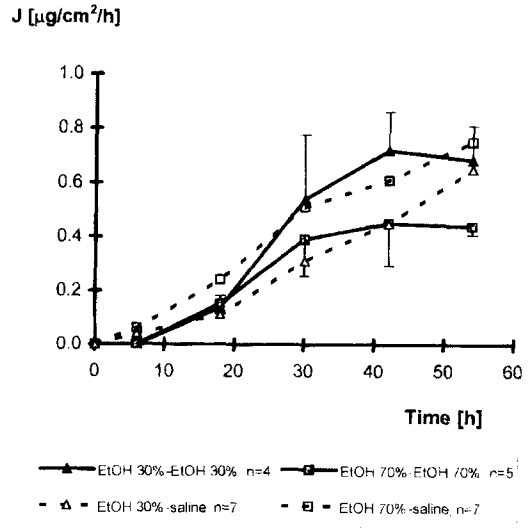

Fig. 3. Penetration rate of baclofen through human skin in vitro from 30% and 70% ethanol when the receiver fluid was saline (asymmetric model) or the appropriate ethanol-water mixture (symmetric model). All data represent the mean ± S.D.

Ethanol causes an increase in the volume of the polar pathway, creating new pores or expanding the existing ones which results in better permeability of the stratum corneum to ions. The present study may be concluded with the following assumptions: ethanol at concentrations up to 70% changes the nature of the porous pathway slowly, while the effect of 95% ethanol is rapid and maximal; the flux of the ionized permeant through the pores depends on its solubility in the vehicle. The dynamics of hydration of the stratum corneum are important for the transport through the pores and further studies should be carried out with special care to control this process.

References

Berner, B. and Cooper, E.R. Models of skin permeability. In Kydonieus, A.F. and Berner, B. (Eds.), *Transdermal Delivery of Drugs*, Vol. 2, CRC Press, Boca Raton, 1987, pp. 42–56.

Berner, B., Mazzenga, G.C., Otte, J.H., Steffens, R.J., Juang, R.-H. and Ebert, C.D. Ethanol:water mutually enhanced transdermal therapeutic system. II: Skin permeation of ethanol and nitroglycerin. *J. Pharm. Sci.*, 78 (1989) 402–407.

Ghanem, A.-H., Mahmoud, H., Higuchi, W.I., Rohr, U.D., Borsadia, S., Liu, P., Fox, J.L. and Good, W.R. The effects of ethanol on the transport of β-estradiol and other permeants in hairless mouse skin. II. A new quantitative approach. In Anderson, J.M. and Kim, S.W. (Eds.), *Advances in Drug Delivery Systems*, 3, Elsevier, Amsterdam, 1987, pp. 75–83.

Ghanem, A.-H., Mahmoud, H., Higuchi, W.I., Liu, P. and Good, W.R. The effect of ethanol on the transport of lipophilic and polar permeants across hairless mouse skin: methods/validation of a novel approach. *Int. J. Pharm.*, 78 (1992) 137–156.

Kurihara-Bergstrom, T., Knutson, K., DeNoble, L.J. and Goates, C.Y. Percutaneous absorption enhancement of an ionic molecule by ethanol-water systems in human skin. *Pharm. Res.*, 7 (1990) 762–766.

Sznitowska, M. and Berner, B. Polar pathway for percutaneous absorption. In Surber, C., Elsner, P. and Bircher, A.J. (Eds), *Exogenous Dermatology, Curr. Prob. Dermatol.*, Vol. 22, Karger, Basel, 1995, pp. 164–170.

Sznitowska, M. and Janicki, S. *In vitro percutaneous permeation of a model zwitterion, baclofen.* Proc. 1st World Meeting APGI/APV, Budapest, 9/11 May 1995.

Sznitowska, M., Berner, B. and Maibach, H.I., In vitro permeation of human skin to multipolar ions. *Int. J. Pharm.*, 99 (1993) 43–49.

Sznitowska, M., Berner, B. and Maibach, H.I. Percutaneous penetration of multipolar ions: evidence for porous transport. *Int. J. Pharm.*, 123 (1995) 41–45.