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International Journal of Pharmaceutics 154 (1997) 141–148

international journal of pharmaceutics

Research papers

The effects of several penetration-enhancers on the simultaneous transport and metabolism of ethyl nicotinate in hairless rat skin

Teruaki Hayashi [a], Yumiko Iida [a], Tomio Hatanaka [a], Takeo Kawaguchi [a], Kenji Sugibayashi [a,b], Yasunori Morimoto [a,b,*]

[a] *Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan*
[b] *Life Science Research Center, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan*

Received 7 December 1996; received in revised form 18 March 1997; accepted 17 April 1997

Abstract

The effects of enhancers such as Azone, L-menthol, ethanol and the L-menthol-ethanol-water (MEW) system on the simultaneous transport and metabolism of ethyl nicotinate (EN) through excised hairless rat skin were measured. The sum of the skin permeability coefficients of EN and its metabolite, nicotinic acid (NA), was utilized to assess their penetration-enhancement abilities, and the flux ratio of NA against the total (EN + NA) flux was used as a metabolic index to evaluate the effect of the enhancers on the skin metabolism of EN. The addition of 1% L-menthol increased the total skin permeability coefficient to about 2.5 times that of the control (without L-menthol), whereas 40% ethanol decreased this coefficient. This was probably due to the lowered thermodynamic activity of EN in the vehicle caused by the addition of ethanol. The simultaneous use of L-menthol and ethanol (MEW system) showed a similar permeability coefficient to that of the control. This may be explained by the decreased activity of EN caused by ethanol and by the increased diffusivity of the skin caused by L-menthol. All of the enhancers decreased the metabolic index in hairless rat skin, compared to the control. The reversibility of this index was then evaluated with a pretreatment experiment of the enhancers. The metabolic activity from EN to NA in skin was immediately recovered after the removal of ethanol, L-menthol or MEW system. The penetration-enhancing effect resulting from 1% L-menthol pretreatment was lower than that resulting from MEW pretreatment or 3% Azone pretreatment. With respect to the metabolic index recovery rate in skin, L-menthol may be much safer than Azone. L-Menthol, ethanol and the MEW system are therefore safe and effective ways to enhance or control skin permeation and to decrease the skin metabolism of prodrugs and peptides which are easily bioconverted in skin. © 1997 Elsevier Science B.V.

Keywords: Skin metabolism; Skin permeation; Ethyl nicotinate; Nicotinic acid; Enhancer; Azone; L-Menthol; Ethanol; MEW system

* Corresponding author.

PII S0378-5173(97)00132-4

1. Introduction

Several chemical enhancers were studied to increase the skin permeation of drugs, and some of them have already been applied in marketed transdermal drug delivery systems (TDDS) (Hille, 1993). Most enhancers act on the stratum corneum, the uppermost layer of skin and the greatest barrier to the overall skin permeation of most drugs, to increase the diffusivity of the skin and to increase the partitioning of drugs within the skin barrier (Yamashita et al., 1993). Several enhancers may penetrate into the viable epidermis and dermis to affect the metabolism or bioconversion of drugs in viable skin (Liu et al., 1991). Prodrugs and peptides which are easily degraded by enzymes in the skin are likely candidates for the new TDDS (Steinsträsser and Merkle, 1995). The investigation of the effect of enhancers on skin metabolism and skin permeation of new drug candidates is therefore very important. Some reports described the effect of chemical enhancers on the drug metabolism in skin (Yano et al., 1991; Higo et al., 1992): They used methyl salicylate and nitroglycerin as substrates, and L-menthol and Azone as enhancers, respectively. The effects of enhancer on the skin metabolism, however, are dependent on substrates as well as the enhancers. In the present study, ethyl nicotinate (EN) was selected as a model drug or prodrug, and Azone (laurocapram) (Sugibayashi et al., 1985), L-menthol, ethanol (Liu et al., 1991) and the L-menthol-ethanol-water system (MEW system) (Morimoto et al., 1993) were selected as enhancers which were well studied on their modes of action. The skin permeation studies of EN with simultaneous use and pretreatment use of the enhancers were then conducted and the effects of enhancers on the skin permeation and metabolism of EN were investigated.

It was found from our previous work using excised hairless rat skin (Sugibayashi et al., 1996) that EN was metabolized into nicotinic acid (NA) in the viable epidermis and dermis and that both EN and NA were observed in the receiver chamber when EN was applied to the skin surface. The ratio of NA flux against total (NA + EN) flux may be a good index to evaluate the effects of enhancers on the skin metabolism of EN. The ratio, however, was dependent upon the EN concentration applied. Then a relationship between the flux ratio and the total flux was adopted to evaluate the metabolic effect of each enhancer.

2. Materials and methods

2.1. Materials

EN, NA, L-menthol and ethanol were obtained from Wako Pure Chemical Industries, (Osaka, Japan). Azone was kindly supplied by Nelson-Sumisho, (Tokyo, Japan). Carbopol 934 was obtained from B.F. Goodrich Chemical (Cleveland, OH, USA). Other chemicals and solvents were of reagent grade and they were used without further purification.

2.2. Animals and skin membrane preparations

Male hairless rats (WBN/IL-Ht), 9 weeks old and weighing 180–220 g, supplied either by Ishikawa Laboratory Animals (Fukaya, Saitama, Japan) or Life Science Research Center, Josai University (Sakado, Saitama), were used in all of the animal experiments. The rats were sacrificed by cervical dislocation before the experiments. The abdominal region of hairless rat skin was then carefully shaved and the full-thickness of this skin was excised before the experiments.

The stripped skin was obtained by stripping the stratum corneum 20 times from the shaved abdominal skin with adhesive cellophane tape (24 mm width, Nichiban Co., Tokyo) (Washitake et al., 1973). A composite consisting of silicone membrane (200 μm in thickness, Silastic®, Dow Corning, Midland, MI, USA) and stripped skin was designed: The silicone membrane was layered on the epidermis side of the stratum corneum-stripped skin to make a silicone membrane-stripped skin composite.

In order to prepare enhancer-pretreated skin, four different kinds of 0.7% Carbopol gels containing Azone (at a concentration of 3%), L-menthol (1%), ethanol (40%) or L-menthol (3%)-ethanol (40%)-water system were applied to

the abdomens of rats for 12 h in vivo. The rats were then sacrificed and the skin was excised from each rat following careful removal of the gel from the abdomens. Plane Carbopol gel (without enhancer) was also used as the control. A detailed method is reported elsewhere (Hosoya et al., 1987).

2.3. In vitro membrane permeation procedure

A side-by-side diffusion cell set consisting of two half cells (Okumura et al., 1989) was used to measure skin permeation of EN and metabolism from EN to NA. A piece of full-thickness skin, silicone membrane-stripped skin composite or enhancer-pretreated skin was mounted between the two half cells, each having 2.5 ml in volume and 0.95 cm^2 in effective diffusion area. The dermis-side of the cell was filled with 2.5 ml of phosphate buffered saline (pH 7.4) and the stratum corneum-side was filled with the same volume of 2.53 to 99.23 μmol/ml EN aqueous solution (with or without enhancer). Both donor and receiver compartments were stirred with a star-head bar driven by a constant-speed synchronous motor (MC-301, Scinics, Tokyo) at about 1200 rpm. The permeation study was conducted at 37°C. The receiver solution was withdrawn every hour over a period of 8 h and analyzed for EN and NA by HPLC, and the same volume of fresh buffer was added to the cell to keep the volume constant.

2.4. HPLC analysis

EN and NA were determined by HPLC which consisted of a pump (LC-6A, Shimadzu, Kyoto, Japan), an ultraviolet detector (SPD-6A, Shimadzu), a 4.6 mm × 250 mm stainless steel column packed with Nucleosil 5C18 (Macherey Nagel, Germany) and an integrator (C-R6A, Shimadzu). The mobile phase consisted of a 1:2 of acetonitrile: 15 mM phosphoric acid solution (pH 2.2) containing 5 mM sodium dodecylsulfate, and the flow rate was 1.0 ml/min. EN and NA were detected using a UV absorbance of 264 nm, and ethyl *p*-hydroxybenzoate was used as an internal standard.

3. Results and discussion

3.1. Establishment of the evaluation method for metabolic activity

The Michaelis constant and the maximum reaction rate for the metabolism of EN to NA in skin, K_m and V_{max}, respectively, can be used as indexes for evaluating the metabolic activity in skin. These parameters in intact skin (skin that was not treated with enhancer) were 837 nmol/ml and 5.70 μmol/cm^2/h, respectively: These parameters were obtained from the hydrolysis experiment of EN using skin homogenate (Sugibayashi et al., 1996). The in vitro skin permeation and metabolism profiles of EN were very consistent with the simulated profiles using these parameter values. When an enhancer was added, however, these parameters were not obtained from the skin homogenate, because the hydrolysis rate of EN to NA gradually decreased over time. This was probably due to stepwise increase in the enhancer inhibition of the enzymatic reaction.

From our previous study (Sugibayashi et al., 1996), the pseudo steady-state flux of NA into the receiver cell was increased to a convergent value (about 5 μmol/cm^2/h) with an increase in the application concentration of EN on skin without enhancer. This saturated NA flux was very close to the V_{max} for the intact skin (5.70 μmol/cm^2/h). When the metabolite flux with an enhancer system is lower than this value, the metabolism of EN to NA in the viable epidermis and dermis may be suppressed. The enhancer, however, may change the permeation of EN across the stratum corneum, generally by increasing it. The effect of the enhancer on the metabolism should thus be modified to take into consideration the extent of skin permeation enhancement.

High concentrations of EN or NA in skin may also affect metabolic activity. In order to evaluate whether skin permeation enhancement affects the metabolic function (K_m and V_{max}) of skin or not, a composite consisting of silicone membrane and stripped skin was designed. Since the composite exhibits much higher permeation of EN than full-thickness skin, it can be used as a membrane model showing a high drug flux without changing

the intrinsic metabolic function of the viable epidermis and dermis. The silicone membrane is also useful in preventing the back-flux of NA produced in viable skin, because the membrane is lipophilic enough to resist metabolite penetration.

Fig. 1 shows the steady-state fluxes of EN, NA and their sum across the silicone membrane-stripped skin composite. The total flux increased in a nearly linear way with respect to the EN to the concentration applied and the total permeability coefficient (sum of the permeability coefficient of EN and NA) was found to be only marginally dose dependent. The permeability coefficient across the composite was $2.10 \pm 0.32 \times 10^{-1}$ cm/h, and this was about 5 times greater than that of full-thickness skin ($4.00 \pm 0.40 \times 10^{-2}$ cm/h). Solid lines in Fig. 1 show the simulated values obtained by solving following partial differential equations under appropriate initial and boundary conditions.

$$\frac{\partial C_{EN}}{\partial t} = D_{SM}\frac{\partial^2 C_{EN}}{\partial x^2} \text{ (in silicone membrane)}$$

$$\frac{\partial C_{EN}}{\partial t} = D_{EN}\frac{\partial^2 C_{EN}}{\partial x^2} - \frac{V_{max}\cdot C_{EN}}{K_m + C_{EN}} \text{ (in stripped skin)}$$

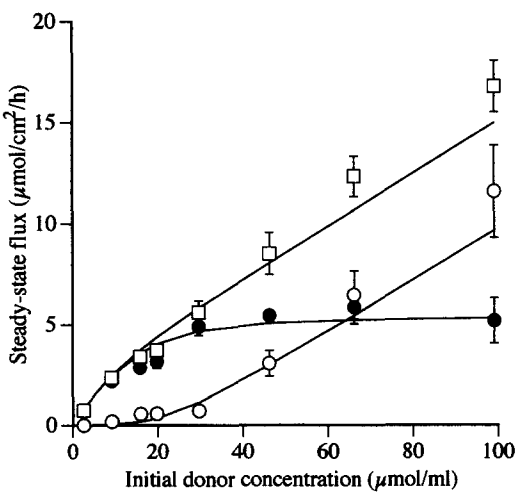

Fig. 1. Relations between the fluxes of ethyl nicotinate, nicotinic acid and their combined flux sum through silicone membrane-stripped skin composite and the initial donor concentration of ethyl nicotinate. (○): ethyl nicotinate, (●): nicotinic acid, (□): total. Solid lines show simulated values. Each point represents the mean ± S.E. of 5 experiments.

$$\frac{\partial C_{NA}}{\partial t} = D_{NA}\frac{\partial^2 C_{NA}}{\partial x^2} + \frac{V_{max}\cdot C_{EN}}{K_m + C_{EN}} \text{ (in stripped skin)}$$

where C_{EN} and C_{NA} are EN and NA concentration at a position of x and time t, and D_{SM}, D_{EN} and D_{NA} are diffusion coefficients of EN in the silicone membrane and of EN and NA in stripped skin, respectively. Relations between EN and NA fluxes and EN concentration applied were well simulated, as the same in the full-thickness skin (Sugibayashi et al., 1996), by the K_m and V_{max} obtained from skin homogenate, permeability coefficient of EN through silicone membrane (0.597 cm/h), and diffusion coefficients of EN and NA in stripped skin (1.40×10^{-2} and 1.99×10^{-2} cm^2/h, respectively, calculated from separate permeation experiments). These results suggest that the enhanced skin delivery of EN and NA did not change the metabolic parameters in skin and that this model membrane is a useful tool which enhances the delivery of EN and NA through viable skin. The solid line for the total flux was, however, a little convex showing that the rate limiting step was no more in the silicone membrane. If the permeation barrier of EN in the silicone membrane was as high as that in the stratum corneum, then the total flux would be proportional to the concentration of EN applied.

Assuming the rate-limiting step of EN skin permeation is the diffusion process in the stratum corneum and no metabolism takes place in this layer, EN metabolism in skin is dependent upon the amount of EN which penetrates into the viable epidermis. With this in mind, we set out to evaluate the effect of enhancers on the metabolism of EN in skin by using a ratio of the amount of NA produced in skin over the amount of EN which was able to penetrate into the viable epidermis from a vehicle (donor solution) through the stratum corneum. Fig. 2 shows the relation between J_{tot} and the J_{NA}/J_{tot} ratio, where J_{tot} and J_{NA} are the total (EN and NA) flux and the NA flux, respectively, across the skin at steady-state. The J_{tot} corresponds with the amount of EN which was able to penetrate into the viable epidermis through the stratum corneum, and the J_{NA}/J_{tot} ratio may be a metabolic index to the amount of NA produced from EN in skin. The solid line in the figure was calculated by the K_m and V_{max}

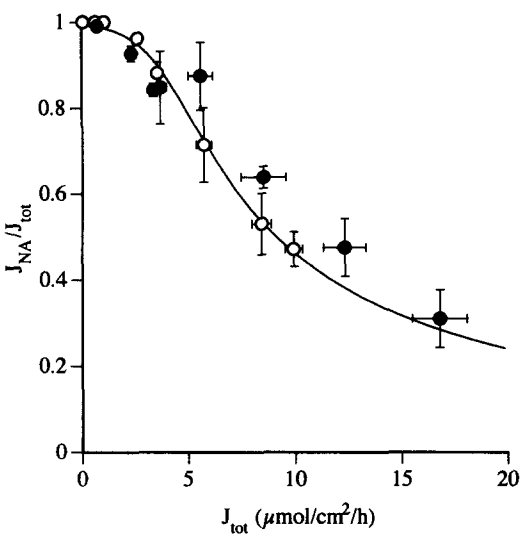

Fig. 2. Relations between the ratio of the nicotinic acid flux versus the total (ethyl nicotinate and nicotinic acid) flux and the total flux through full-thickness skin and silicone membrane-stripped skin composite. (○): full-thickness skin, (●): silicone membrane-stripped skin composite. Applied EN concentrations were 1.455–231.5 μmol/ml for full-thickness skin and 2.534–99.23 μmol/ml for silicone membrane-stripped skin composite. Solid lines show calculated values using K_m and V_{max} without an enhancer. Each point represents the mean ± S.E. of 5 experiments.

obtained from the hydrolysis experiment using skin homogenate and the diffusion coefficients of EN and NA in the viable epidermis and dermis were calculated from separate permeation experiments. This line indicates that the metabolic function in skin was not damaged. Almost the same line for the full-thickness skin with that for silicone membrane-stripped skin composite again suggests that permeation enhancement itself is independent of the change in metabolic activity of skin.

3.2. The simultaneous treatment of EN and enhancer in vitro

3.2.1. The effect on skin permeation

EN was metabolized to NA in skin and no other metabolites were found. NA was not also detected in donor solution throughout the experiments. The sum of the EN and NA fluxes or permeability coefficients was then used to assess an enhancement ability of the additives. Fig. 3 shows the effect of several enhancers on the total permeability coefficient. The permeability coefficients were represented as the mean of all experimental values obtained for different concentrations of EN applied, because the permeabilities were not dependent on the applied EN concentration. When 3% Azone was applied, no skin permeations of EN and NA were observed, probably due to the high affinity of EN in Azone droplets in the donor chamber (compared to the low affinity of EN in the stratum corneum). The addition of L-menthol at a concentration of 1% in EN solution increased the total permeability to 2.5 times that of the control (which had no enhancer). In contrast, ethanol (40%) decreased the coefficient by 75%. This was probably due to the lowered thermodynamic activity of EN in the vehicle caused by the addition of ethanol. The affinity of EN and a solvent may be evaluated by using the EN solubility level in the solvent: that in water at 37°C is 44 mg/ml; and that in ethanol is infinitely soluble. Ethanol usually increases flux, but it decreases the permeability coefficient of a lipophilic compound like EN from several saturated solutions of the drug. The permeability coefficient from the MEW system (1% L-menthol and 40% ethanol) was similar to the control value.

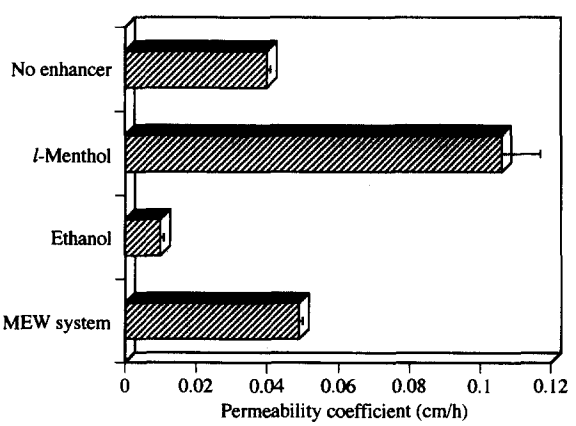

Fig. 3. The effect of penetration enhancers on the total (ethyl nicotinate and nicotinic acid) permeability coefficient through full-thickness skin. Each column represents the mean ± S.E. of 5 experiments.

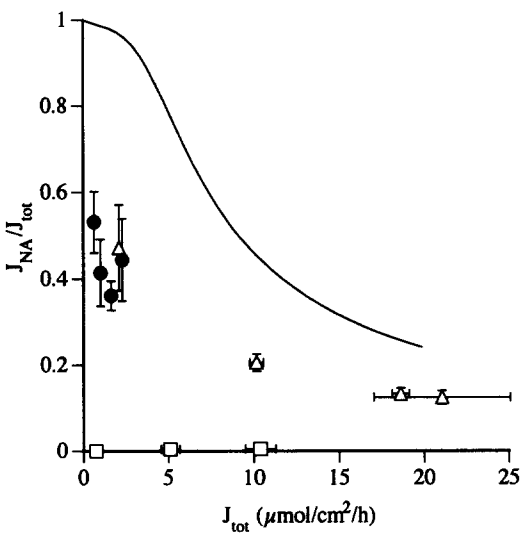

Fig. 4. The effect of penetration enhancers on the metabolism of ethyl nicotinate to nicotinic acid in skin. ——: no enhancer, (△): L-menthol, (●): ethanol, (□): MEW system. Applied EN concentrations were 15.22–231.5 μmol/ml for L-menthol, 72.77–264.6 μmol/ml for ethanol and 14.55–199.8 μmol/ml for MEW system. Each point represents the mean ± S.E. of 5 experiments.

This may be explained by the decreased activity of EN caused by ethanol and the increased diffusivity of EN in skin caused by L-menthol.

3.2.2. The metabolic effect

Fig. 4 illustrates the effect of the enhancers on the relation between J_{tot} and the J_{NA}/J_{tot} ratio. No data points were shown for Azone, for the same reason given above. L-Menthol, ethanol and the MEW systems decreased the enzymatic activity hindering the hydrolysis of EN to NA in skin, because every point was much lower in value than those on the solid line which show no change in activity. A marginal NA flux was observed with the MEW system, whereas the NA flux was reduced by 50% following treatment with L-menthol or ethanol, compared to the control (which had no enhancer). Therefore the simultaneous treatment of the enhancer and EN decreased the metabolic activity, although the decrement varied between the enhancers.

3.3. Enhancer treatment prior to EN application on the skin (enhancer pretreatment)

Since the enhancers decreased the enzymatic activity in skin and lowered the ratio of NA flux versus the total flux, the reversibility of the hydrolysis activity in skin was then evaluated with enhancer-pretreated skin. Namely, the in vitro skin permeation experiment of EN was carried out without the enhancers using excised abdominal skin which was pretreated with the enhancers for 12 h in vivo.

3.3.1. The effect on skin permeation

Fig. 5 shows the effect of enhancer pretreatment on the total permeability across the skin. No significant difference in the permeability coefficients was found between the plane gel pretreatment trial (without enhancer) and the non-pretreatment trial (with no enhancer in Fig. 3). Azone pretreatment increased the permeability coefficient by 275% compared with the plane gel, whereas L-menthol did not. Because L-menthol increased the permeability during simultaneous treatment (Fig. 3), coexistence of the enhancer with drugs is necessary for an enhancing effect, in a similar way as the monoglycerides (Okumura et al., 1990). Ethanol pretreatment slightly increased the permeability. In this case, apparently, no de-

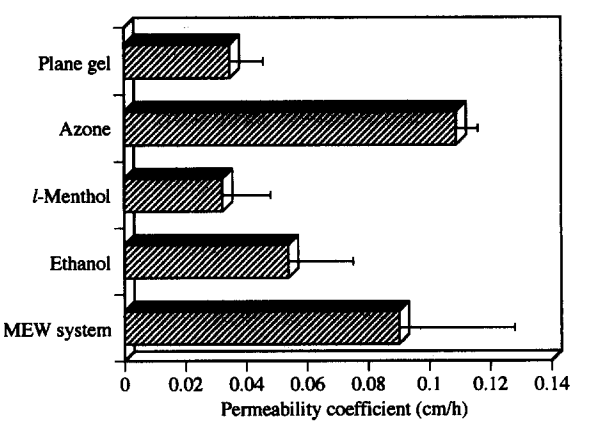

Fig. 5. The effect of penetration enhancer pretreatment on the total (ethyl nicotinate and nicotinic acid) permeability coefficient through full-thickness skin. Each column represents the mean ± S.E. of 5 experiments.

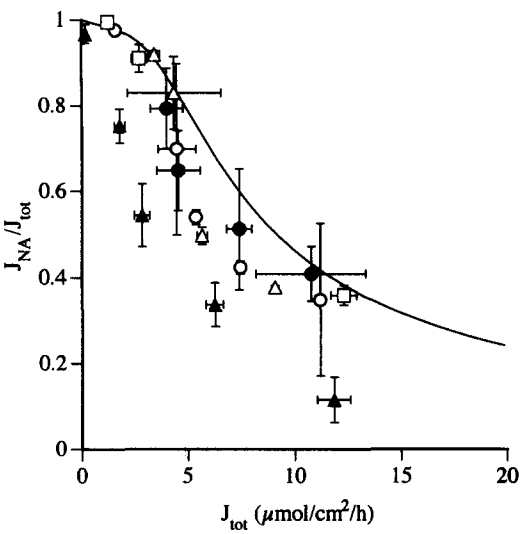

Fig. 6. The effect of penetration enhancer pretreatment on the metabolism of ethyl nicotinate to nicotinic acid in skin. ——: no enhancer (without pretreatment, the same as in Fig. 4), (○): plane gel, (▲): Azone, (△): L-menthol, (●): ethanol, (□): MEW system. Applied EN concentrations were 72.77–250.0 μmol/ml for plane gel, 1.460–105.9 μmol/ml for Azone, 72.77–250.0 μmol/ml for L-menthol, 72.77–231.5 μmol/ml for ethanol and 25.00–72.77 μmol/ml for MEW system. Each point represents the mean ± S.E. of 5 experiments.

crease in the thermodynamic activity of EN in the vehicle took place. Interestingly, the simultaneous use of L-menthol and ethanol, i.e. the MEW system, increased the permeability to about 2.6 times that of the control. An increase in L-menthol uptake into the skin barrier by ethanol (Sugibayashi et al., 1995) may be related to the greater permeability coefficient.

3.3.2. The metabolic effect

Fig. 6 illustrates the effect of enhancer pretreatment on EN metabolism. Azone pretreatment obviously suppressed the metabolic reaction, because every point for the Azone pretreatment was much lower than the data for plane gel. A similar finding was reported for the skin delivery of nitroglycerin (Higo et al., 1992). When a wash out period (1 or 2 days) for Azone was administered, the metabolic activity against EN was recovered (data not shown). We have already reported that this type of wash out period decreases the enhancing effect of Azone on skin penetration (Adachi et al., 1988) and we suggested that the effect might be related to its concentration in the skin barrier (stratum corneum). Metabolic activity in skin may be dependent on the enhancer content in the viable skin tissue. Pretreatment with L-menthol, ethanol and the MEW system, however, did not significantly decrease the metabolic reaction rate, although their simultaneous treatments did suppress the reaction (Fig. 4).

The reason why metabolic ratio for the plane gel pretreatment was lower than the solid line (without pretreatment) is not clear. Similar values for the pretreatment with L-menthol, ethanol and the MEW system to that of the plane gel, however, suggest that the metabolic effects of these enhancers are reversible. The enhancing effect of 1% L-menthol was lower than that of 3% Azone (Fig. 5), but the former may be much safer than the latter in terms of the recovery rate of metabolic activity in skin.

4. Conclusion

The effect of enhancers on the simultaneous diffusion and metabolism of EN in skin was evaluated. The index for EN metabolism in skin, J_{NA}/J_{tot}, was a function of J_{tot}. These relations were used to evaluate the effects of enhancers on skin metabolic activity. Azone, L-menthol, ethanol and the MEW system decreased the metabolic activity of EN in skin, but the rank order of the decreased metabolic rate was not consistent with that in the decreased permeation rate. Ethanol, L-menthol and the MEW system displayed great reversibility in their effect on EN metabolism in skin. These enhancers are therefore useful for increasing skin permeation and decreasing skin metabolism of prodrugs and peptides which are easily bioconverted in skin. Skin contains many kinds of enzymes and our approach against esterases for EN hydration may be applied to other enzymes in skin.

Acknowledgements

Part of this research was supported by Japan Research Foundation for Clinical Pharmacology.

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