[Chem. Pharm. Bull. 36(4)1519—1528(1988)]

Effect of Several Penetration Enhancers on the Percutaneous Absorption of Indomethacin in Hairless Rats[1)]

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(Received October 7, 1987)

In order to measure the effect of several penetration enhancers on the percutaneous absorption of drugs and to classify the enhancers into several categories in terms of the mode and mechanism of action, *in vitro* permeation experiments with a model drug, indomethacin, from aqueous, ethanolic and binary solvent systems were carried out by using excised hairless rat abdominal skin and a 2-chamber diffusion cell at 37 °C. Among the enhancers used in the present experiments, Azone, dimethyl sulfoxide (DMSO), isopropylmyristate, diethylsebacate, diethylmethylbenzamide, methylpyrrolidone and salicyclic acid enhanced the skin permeation of indomethacin, whereas urea, pyrrolidonecarboxylic acid and sodium hyaluronate did not as compared with the same solvent system without enhancer. These results suggest that the lipophilicity of enhancers may be an important factor for the penetration-enhancing effect on the skin permeation of indomethacin. It is also suggested that the effect of methylpyrrolidone is dependent upon the kind of solvent system; higher water content resulted in a lower effect.

Further experiments to clarify the mechanism of action were done with pyrrolidones and salicylates, since it has already been reported that other enhancers such as Azone and DMSO might mainly affect the lipid in skin. Pyrrolidones had an *in vitro* moisturizing effect on keratin tablets and an *in vivo* skin-moisturizing effect. They affected the dissolution of keratin powder. On the other hand, salicylates dissolved and softened keratin powder, but they had no effect on the skin moisturizing. These results suggest that pyrrolidones and salicylates affect the keratin cells in the stratum corneum to enhance skin permeability, although the mechanism of action is different between the two groups.

Keywords——penetration enhancer; percutaneous absorption; indomethacin; hairless rat; salicylate; pyrrolidone

Introduction

The absorption rate of drugs through the skin is generally much slower than that through the gastro-intestinal tract. In order to overcome the low bioavailability, improved dermal delivery of drugs has been the focus of pharmaceutical research worldwide for many years. One of the goals is to find a substance of low toxicity which is non-irritating and which can deliver a wide variety of compounds effectively.

In the present study, the effect of several penetration enhancers on the percutaneous absorption of a drug was measured by the *in vitro* method and the enhancers were classified to several categories in terms of the mode and mechanism of action. Indomethacin (IND) was selected as a model compound since it has been widely used, systemically and/or locally, as an anti-inflammatory, anti-pyretic agent for transdermal delivery. Most *in vitro* permeation experiments were done by using IND suspensions to ensure equal thermodynamic activity of IND in the drug-donor compartment.

Experimental

Materials——IND, flufenamic acid and salicylic acid (SA) were commercial products of JP grade. Azone (1-

dodecylazacycloheptan-2-one, laurocapram), glycol salicylate (GS) and sodium hyaluronate (SH) were kindly supplied by Nelson-Sumisho Co. (Tokyo, Japan), Lion Corp. (Tokyo) and Shiseido Co. (Tokyo), respectively. Other penetration enhancers such as dimethyl sulfoxide (DMSO), urea, pyrrolidone (P), and propyl salicylate (PS) were obtained from Wako Pure Chemical Industries (Osaka, Japan), and enhancers such as isopropyl myristate (IPM), diethylsebaçate (DES), *N,N*-diethyl-3-methylbenzamide (Deet), *N*-methylpyrrolidone (MP), *N*-ethylpyrrolidone (EP), 2-pyrrolidone carboxylic acid (PC), methyl salicylate (MS), and ethyl salicylate (ES) were from Tokyo Kasei Kogyo Co. (Tokyo). Keratin powder (Tokyo Kasei Kogyo), Carbopol 934 (B. F. Goodrich Chemical Co., Cleveland, OH, U.S.A.), hydroxypropylcellulose-M (Wako Pure Chemical Industries) and Ethomeen C-25 (Lion-Akzo Corp., Tokyo) were used. Other chemicals and solvents were of reagent grade quality and used without further purification.

Animals——Male hairless rats (WBN/kob strain) weighing about 150 g, supplied by Saitama Laboratory Animals (Sugito, Saitama, Japan), were used in all animal experiments.

Procedure for *in Vitro* Skin Permeation of IND——The abdominal region of hairless rat skin, which had been shaved with an electric razor, was excised and mounted between two half cells, each having 2.0 ml volume and 0.636 cm^2 effective diffusion area.[2] The donor compartment (facing to the stratum corneum) was filled with 2 ml of drug suspension (about 2—3 times higher concentration than the solubility of IND in each solvent) containing a penetration enhancer in either water, ethanol, or water–ethanol binary mixed solvent (40% EtOH). The concentration of penetration enhancer was chosen so as to obtain a suspension in each solvent. Ten percent was used generally. The receiver compartment (facing to the dermis) was filled with 2 ml of isotonic phosphate buffer (pH 7.4). The experiments were done in a water bath at 37 °C. The donor and receiver compartments were mixed throughout the experiment with Teflon stirrers driven by a 150 rpm constant-speed motor. At appropriate times, 100 μl samples were withdrawn from the receiver compartment for analysis. The same volume of the same buffer was added to the receiver compartment to keep the volume constant.

Analysis of IND——Methanol (100 μl) containing flufenamic acid as an internal standard was mixed with the sample solution (100 μl) and centrifuged at 16000 rpm for 5 min for deproteinization. The resultant supernatant was injected into an high performance liquid chromatography (HPLC) system (LC-6A, Shimadzu, Kyoto, Japan). HPLC conditions were as follows: column, 4.6 mm × 250 mm stainless steel column packed with Nucleosil $5C_{18}$ (Macherey Nagel, Germany), elution solvent, methanol: 0.02 M KH_2PO_4 (3 : 1), detector, ultraviolet (UV) 262 nm. Typical retention times of flufenamic acid and IND were 7.3 and 9.4 min, respectively.

Measurement of *in Vitro* Keratin Moisturizing——Ten microliters (in the case of a liquid) or milligrams (in the case of a solid) of a pyrrolidone or salicylate was mixed thoroughly with 100 mg of keratin powder. The resulting powder was directly pressed at 280 kg/cm^2 under reduced pressure in a tablet press for infrared (IR) measurement (Shimadzu SSP-10). After being dried under reduced pressure, the keratin tablets were kept in a chamber at 100% relative humidity at 37 °C for 24 h and then weighed. The water-saturated tablets were moved to another chamber of 80% relative humidity at 37 °C. The time course of loss in tablet weight was followed for 24 h.

Measurement of *in Vivo* Skin-Moisturizing Effect——Carbopol 934 (80 mg), Ethomeen C-25 (160 mg) and hydroxypropylcellulose-M (300 mg) were added to 10 ml of methanol solution containing a pyrrolidone or salicylate at a concentration of 10% to form a gel. This gel (2 g) with a backing of Catherie-pad (Nichiban Co., Tokyo) was applied to 4 × 5.5 cm^2 of the abdominal skin of hairless rats over 24 h.[3] At appropriate times, the electric conductance of the skin surface was measured by using a capacitance conductance meter (IB-355, IBS Co., Hamamatsu, Japan) immediately after removing the gel pad.[4]

Measurement of the Dissolution of Keratin Powder——Keratin powder (10 mg) was added to 20 ml of 40% EtOH containing SA, MS, ES or GS at a concentration of 5%. The resulting keratin suspension was incubated in a water bath at 37 °C. At appropriate times, 2 ml of the suspension was sampled and the concentration of keratin dissolved in the supernatant was measured by a modified Lowry method.[5]

Results

Penetration-enhancer free water, ethanol (EtOH) or 40% ethanol aqueous solution (40% EtOH) was used as the donor solution for control groups. Figure 1 shows the skin permeation of IND from these IND suspensions. The rank order of the skin permeation was EtOH > 40% EtOH > water. Although most profiles of skin permeation of penetrants are found to show a lag period followed by a steady-state permeation rate,[6] the time course of cumulative amount of IND permeated through the skin from ethanol suspension shows a bend at about 18 h. In contrast, the profiles from aqueous and 40% EtOH suspensions show a typical lag time followed by an almost constant rate.

Figure 2 shows the effect of an aprotic solvent, DMSO, and the related compounds, urea and Deet, on IND permeation through skin. Ethanol aqueous solution (40% EtOH) was used

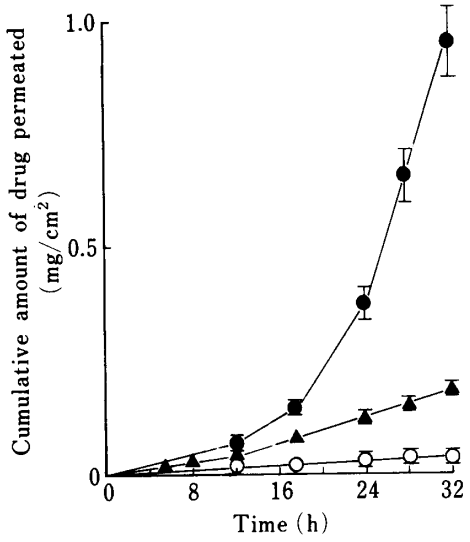

Fig. 1. Time Course of Cumulative Amount of IND Permeated across the Excised Hairless Rat Skin from Three Solvent Systems

Water (○), 40% EtOH (▲), EtOH (●).
Each value represents the mean ± S.E. of at least 3 experiments.

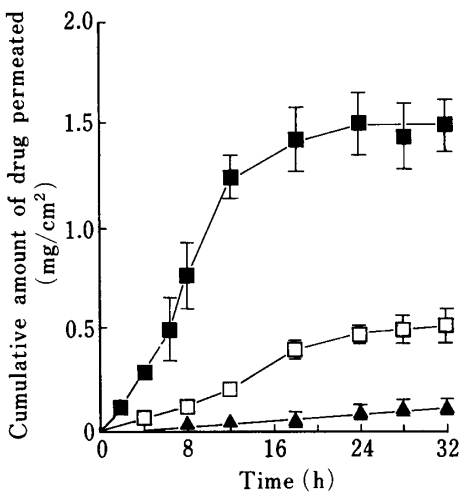

Fig. 2. Effect of DMSO and Related Compounds on the Permeation of IND across the Excised Hairless Rat Skin

DMSO : 40% EtOH = 80 : 20 (■). Urea: 40% EtOH = 10 : 90 (▲). Deet : 40% EtOH = 5 : 95 (□).
Each value represents the mean ± S.E. of at least 3 experiments.

as the donor solution in these experiments. The concentration of the enhancers was set at 80, 5 or 10% for DMSO, urea or Deet, respectively, on the basis of their solubilities in 40% EtOH. The concentrations of enhancers were chosen on a similar basis hereafter. DMSO (DMSO : 40% EtOH = 80 : 20) markedly enhanced the IND permeation compared to the control (40% EtOH). The curve for DMSO treatment showed a bend at about 12 h and reached a plateau after 18 h. This phenomena probably ocurred due to the saturation of IND dissolution in the receiver compartment because of the fast permeation. This view is supported by the fact that the solubility of IND in water is 19 μg/ml at 37 °C. As the permeation rate in Deet treatment (Deet : 40% EtOH = 5 : 95) gradually increased, Deet may take some time to affect the skin barrier. The difference from the corresponding control experiment (40% EtOH as shown in Fig. 1) was about 2-fold at 12 h, but about 3-fold at 24 h. Since the solubility of IND in the receiver solvent would be affected by permeation of donor solvent (EtOH) and enhancers, the reason why the curve for Deet treatment was bent may be the case of DMSO treatment. On the other hand, urea (urea : 40% EtOH = 10 : 90) showed no difference from the corresponding control profile.

Figure 3 shows the effect of Azone, two esters (IPM and DES) and SH. Azone (Azone : 40% EtOH = 3 : 97) markedly enhanced the skin permeation and the effect was a little greater than that in DMSO treatment. The time course of IND permeation with Azone, however, was similar to that with DMSO. A similar permeation profile was also obtained with DES (DES : 40% EtOH = 1 : 99), although the rate and amount of IND permeated were about a half compared to those in the case of Azone treatment. In contrast, IPM treatment (IPM : EtOH = 10 : 90) showed an upward bend in the cumulative amount of IND permeated. Pure ethanol was used as a donor solvent in the IMP experiment because of the immiscibility of IPM in 40% EtOH. The time course with IPM was similar to that of IPM-free ethanol, as shown in Fig. 1. However, the time course of cumulative amount of IND which permeated in the case of IPM treatment was about 10 times higher than that in the control experiment. SH (0.5%) was suspended in water because its solubility in water was higher than that in 40% EtOH or pure EtOH. Although SH was reported to have water-holding capacity in skin,[7] the

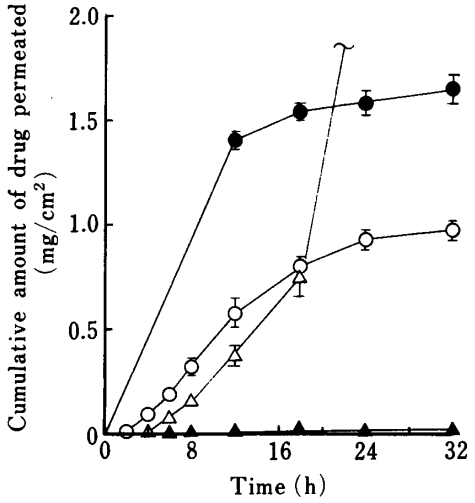

Fig. 3. Effect of Azone, IPM, DES and SH on the Permeation of IND across the Excised Hairless Rat Skin

Azone: 40% EtOH = 3:97 (●). IPM: EtOH = 10:90 (△). DES: 40% EtOH = 1:99 (○). SH: water = 0.5:99.5 (▲).

Each value represents the mean ± S.E. of at least 3 experiments.

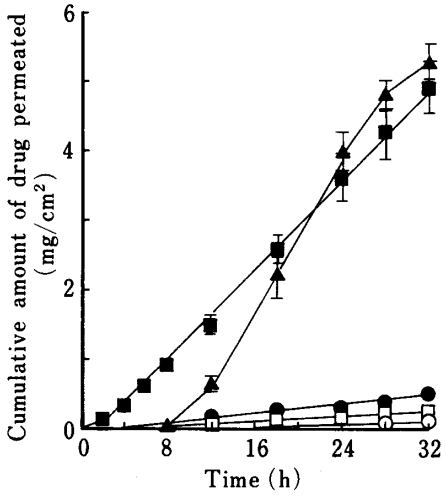

Fig. 4. Effect of Pyrrolidones on the Permeation of IND across the Excised Hairless Rat Skin

MP alone (■). MP: EtOH = 50:50 (▲). MP: 40% EtOH = 50:50 (●). MP: water = 50:50 (□). PC: water = 5:95 (○).

Each value represents the mean ± S.E. of at least 3 experiments.

SH suspension had no enhancing effect on the skin permeation of IND.

Figure 4 shows the effects of MP and PC on the skin permeation of IND, and also shows the effect of the donor solvent in MP treatment on the skin permeation. A high enhancing effect was observed with pure MP. The effect was much higher than that with pure ethanol. The time course of cumulative amount of IND permeated in pure MP treatment was almost linear, whereas a bend was found in the case of pure ethanol treatment. MP diluted with pure ethanol (MP: EtOH = 50:50) showed almost the same enhancing effect as pure MP, although there was a larger lag time. However, MP diluted with 40% EtOH (MP: EtOH = 50:50) or water (MP: H_2O = 50:50) had essentially no effect compared to MP diluted with ethanol. However, MP diluted with 40% EtOH or water showed a greater effect than each MP-free solvent, as shown in Fig. 1. PC (PC: water = 5:95) did not have a penetration-enhancing effect.

As is clear from Fig. 4, the effect of MP is dependent on the solvent used in the donor compartment, and so further experiments were carried out to examine the effects of the donor solvent and concentration of MP on the skin permeation. Figure 5 shows the influence of solvent systems at various concentrations of MP. Cumulative amount of IND permeated per unit application area over 32 h was selected as a parameter of the skin permeation. When MP was diluted twice with ethanol (water free), the effect of MP was almost the same as that of pure MP. When MP was diluted with 40% EtOH or water, however, the effect was greatly reduced. The decrease of the penetration-enhancing effect was the largest when MP was diluted with water, followed by that with 40% EtOH. Water content in the solvents may be an important factor affecting the skin permeation of IND with MP.

Figure 6 shows the effect of SA and GS on the skin permeation. For these experiments, pure ethanol was used as a donor solvent. Large lag times of about 10 and 20 h were found with SA and GS, respectively. Increasing the GS concentration from 10 to 30% increased the skin permeation.

The above results cover for the effects of single enhancers on the *in vitro* permeation of IND through the excised hairless rat skin. Further experiments to clarify the mode and

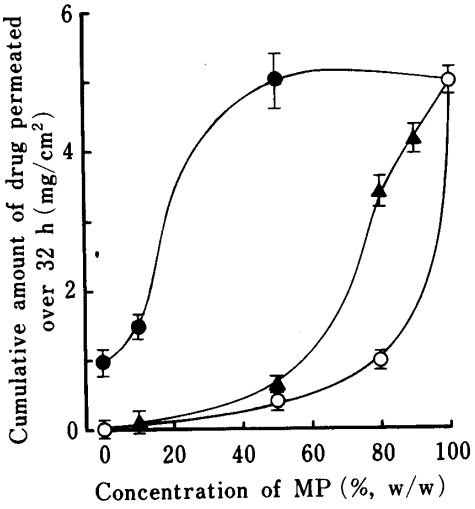

Fig. 5. Effect of Solvent Systems Containing MP on the Cumulative Amount of IND Permeation across the Excised Hairless Rat Skin

Water (○), 40% EtOH (▲), EtOH (●).
Each value represents the mean ± S.E. of least 3 experiments.

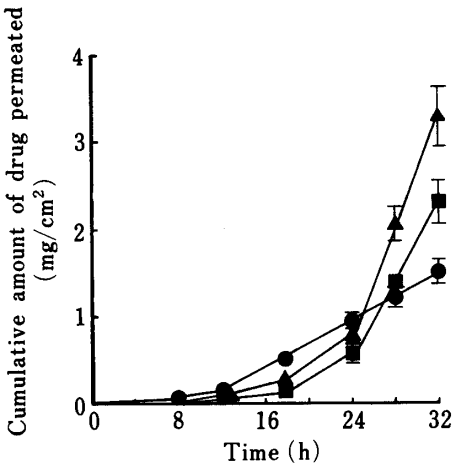

Fig. 6. Effect of Salicylates on the Permeation of IND across the Excised Hairless Rat Skin

SA : EtOH = 10 : 90 (●). GS : EtOH = 10 : 90 (■). GS : EtOH = 30 : 70 (▲).
Each value represents the mean ± S.E. of at least 3 experiments.

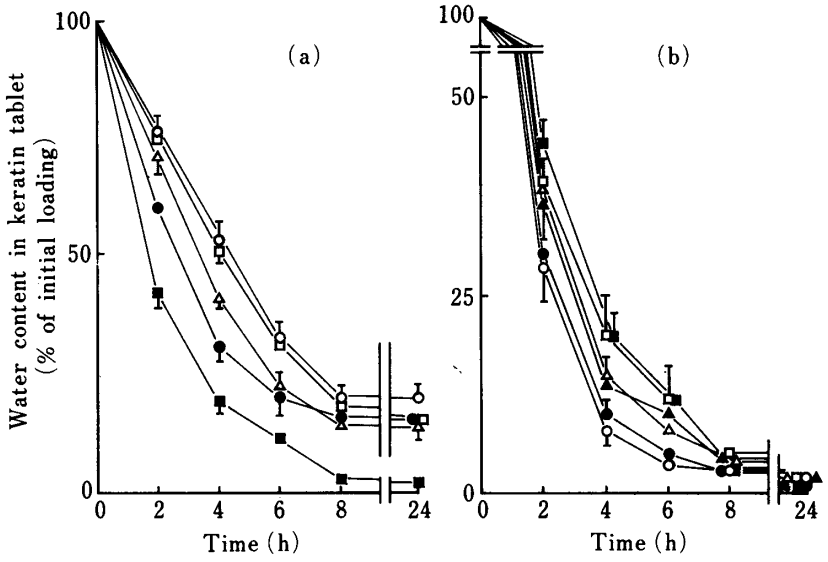

Fig. 7. *In Vitro* Keratin-Moisturizing Effect of Pyrrolidones (a) and Salicylates (b)

(a) Control (■), P (□), MP (○), EP (△), PC (●). (b) Control (■), SA (□), MS (△), ES (●), PS (○), GS (▲).
Each value represents the mean ± S.E. of at least 3 experiments.

mechanism of action of penetration enhancers were done with pyrrolidones and salicylates, since Azone[2,3] and IPM[8] have been investigated separately in our laboratory, while DMSO has been studied by many researchers,[9] DES was studied in combination with IND by Inagi *et al.*[10] and other enhancers, *i.e.* urea, Deet and SH, have little or no effect on the skin permeation of IND as described above.

Figure 7 shows the moisturizing effect of pyrrolidones and salicylates on keratin tablets *in vitro*. The weight of water-saturated keratin tablets decreased with time due to the evaporation of water after a change of the relative humidity from 100 to 80%. Water contents (mean ± S.E.) of the enhancer-free tablets (control tablets) were 44 ± 3, 20 ± 3, 12 ± 1, 3 ± 1 and 2 ± 1 mg per tablet (100 mg initial weight) at 2, 4, 6, 8 and 24 h, respectively. The rates of

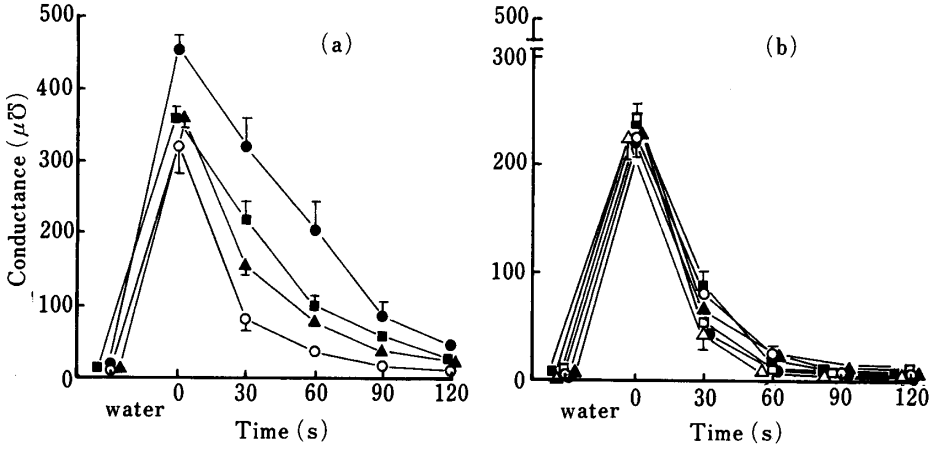

Fig. 8. *In Vitro* Skin-Moisturizing Effect of Pyrrolidones (a) and Salicylates (b)

(a) Control (○), P (▲), MP (●), EP (■). (b) Control (○), SA (□), MS (△), ES (●), PS (■), GS (▲).
Each value represents the mean ± S.E. of at least 3 experiments.

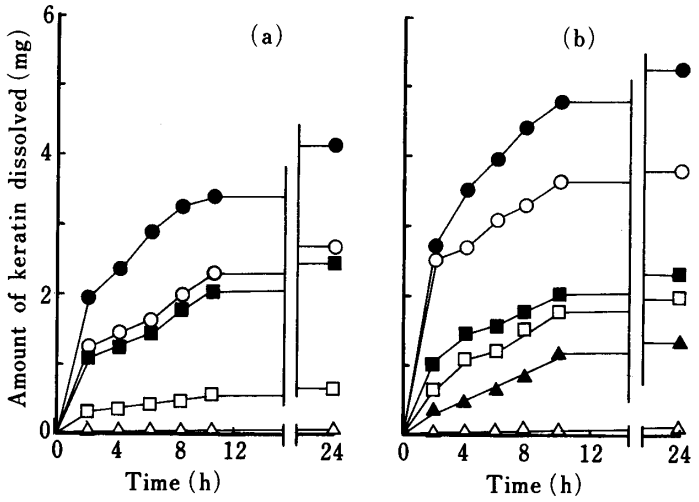

Fig. 9. Effect of Pyrrolidones (a) and Salicylates (b) on the Dissolution of Keratin Powder

(a) Control (△), P (○), MP (●), EP (■), PC (□). (b) Control (△), SA (●), MS (■), ES (□), PS (▲), GS (○).
Each value represents the mean of at least 3 experiments.

decrease of the water content in salicylate-loaded keratin tablets were all faster than that of the control, whereas the rates of all pyrrolidone-loaded tablets were slower than that of the control. These results suggest that pyrrolidones have water-holding capacity. The effect of MP and P was the highest, followed by EP, then PC. The rank order of MP and PC for the water-holding capacity was the same as the effect on the skin permeation as shown in Fig. 4.

In order to confirm the water-holding capacity of pyrrolidones, the electric conductance of the skin surface of the abdominal site in hairless rats treated with methanolic gels containing the enhancers was measured in the *in vivo* experiments. The conductance value increased almost proportionally with the water content on the skin surface.[11] Figure 8 shows the moisturizing effect of pyrrolidones and salicylates on the skin *in vivo*. After treatment with enhancer-free methanolic gel (control), the conductance value was almost zero. The value markedly increased to 220 μ℧ immediately after 10 μl of water was dropped on the skin surface, which demonstrates hygroscopicity of the skin.[4] The conductance decreased every 30 s after that and became almost zero again at 120 s. All salicylates measured in this

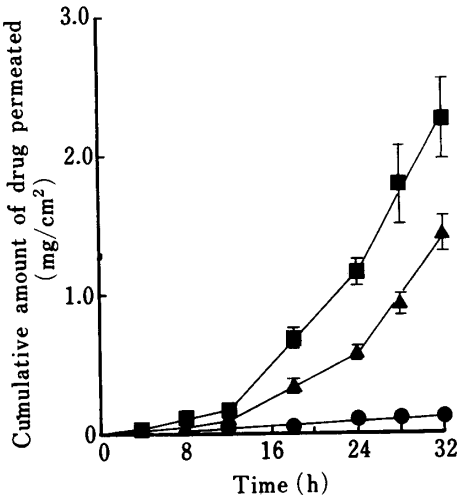

Fig. 10. Effect of Simultaneous Use of Pyrrolidones and Salicylates on the Permeation of IND across the Excised Hairless Rat Skin

SA : GS : EtOH = 5 : 5 : 90 (■). MP : SA : EtOH = 10 : 10 : 80 (▲). MP : PC : water = 5 : 2.5 : 92.5 (●). Each value represents the mean ± S.E. of at least 3 experiments.

experiment showed no difference from the control. On the other hand, pyrrolidones affected the hygroscopicity and water-holding capacity of the skin. MP showed a significantly greater effect than the control from 0 to 90 s.

Figure 9 shows the effect of pyrrolidones and salicylates on the dissolution of keratin powder. The control experiment using enhancer-free 40% EtOH showed no effect on the dissolution of keratin, whereas every enhancer used in this experiment showed a dissolving ability. SA, GS and MP had marked effects among the enhancers tested in the present experiment. The dissolution rate decreased with time in each case, and more than 80% of keratin was dissolved within 10 h relative to the amount of keratin dissolved over 24 h in each experiment.

The effect of combined use of enhancers on the skin permeation was investigated. Three combinations of pyrrolidones (MP+PC), salicylates (SA+GS) and a pyrrolidone and a salicylate (MP+SA) were tested (Fig. 10). As compared with single use of SA (SA : EtOH = 10 : 90) or GS (GS : EtOH = 10 : 90) (Fig. 6), the combined use of SA and GS (SA : GS : EtOH = 5 : 5 : 90) showed no synergism. However, the permeation over 24 h was higher than that in the case of single use of SA and the permeation from 24 to 32 h was higher than that in the case of single use of GA. Simultaneous use of MP with PC (MP : PC : water = 5 : 2.5 : 92.5) or SA (MP : SA : EtOH = 10 : 10 : 80) had no greater effect than single use of MP (MP : EtOH = 10 : 90) (Fig. 4).

Discussion

In the last two decades, a wide variety of compounds have been evaluated as penetration enhancers for transdermal delivery of various drugs. Aprotic solvents,[9,12] *e.g.*, DMSO, *N*,*N*-dimethylacetamide and *N*,*N*-dimethylformamide, and some surface-active compounds[13] were recognized classically as strong enhancers. Recently, pyrrolidones,[14] Deet,[15] salicylates[16] and Azone[2,3,17] have been reported as exceptionally effective enhancers. Some of them, and other typical enhancers, were used in the present experiments.

It is very difficult to evaluate and compare the penetration-enhancing effects of several compounds under fixed conditions. IND was used as a model drug and all experiments were done using IND suspension to ensure equal thermodynamic activity of IND in the donor compartment. The action of penetration enhancers is likely to be strongly dependent on the solvent used in the donor compartment. There are some enhancers which are effective in hydrophilic solutions, whereas others act in lipophilic solutions. It is not always a good experimental design to use a single solvent system for the drug-donor vehicle. Ethanol and

water binary solvent at an ethanol concentration of 40% was mainly used as a donor solution in the present experiment because of the high solubility of most enhancers in it. However, pure ethanol and water were also used instead of 40% EtOH, depending upon the affinity and solubility of the selected enhancers. The concentration of each enhancer was varied in accordance with its solubility in the donor solvent.

Figure 1 shows the results for control groups. There are several reports where a steady-state flux was used for the evaluation of enhancing effect.[18] Since the skin barrier function for drug permeation is not constant, several parameters, such as partition coefficient of drugs from the drug donor to the skin barrier, diffusivity and thermodynamic activity of drugs in the skin barrier and thickness of the skin barrier,[19] change with time after administration of topical formulations. A steady-state flux thus could not be obtained, especially for EtOH treatment, as shown in Fig. 1. The time course of IND permeation through the skin together with the cumulative amount of IND permeated were used for evaluation and comparison of enhancers. Ethanol itself could be thought of as a strong enhancer for IND permeation, as shown in Fig. 1. The effects of ethanol and its concentration on IND permeation are very complicated. The effects might be related to the skin penetration of ethanol itself,[20] and detailed experiments are under way in our laboratory.

Aprotic solvents such as DMSO are known to be strong hydrogen-bonding acceptors, and their hydrogen bonding abilities are considered to be related to their effectiveness as skin penetration enhancers.[21] Azone, urea, Deet and pyrrolidones also have strong hydrogen-accepting abilities. The chemical structures of all these compounds can be represented as either $R_1R_2S=O$ or $R_1R_2NC=OR_3$. These enhancers may interact with constituents of the skin barrier. Among these enhancers, DMSO, Deet, Azone and MP were effective in the skin permeation of IND, whereas urea and PC were not (Figs. 2—4). Since only urea and PC are practically insoluble in chloroform and other organic solvents among the enhancers, lipophilicity seems an important factor in enhancing the skin permeation of lipophilic compounds such as IND. In addition, water content in the vehicle may be one of the most important factors especially in the case of MP (Fig. 5). Similar results were reported for DMSO.[22]

The skin barrier is mainly in the stratum corneum, which is composed of dead epidermal cells derived from living epidermal cells underneath the stratum corneum. The stratum corneum has a multilamellar structure in which keratinized-protein-rich intracellular layers and lipid-rich intercellular layers exist alternately.[23]

Lipophilic compounds such as IPM and DES would affect the lipid-rich phase in the stratum corneum (Fig. 3). Some surfactants might also enhance the drug permeation due to an effect on the stratum corneum lipid.

The skin moisturizing test using keratin tablets may be a good method for evaluating hygroscopicity and water-holding capacity of the keratinized protein in the stratum corneum. Keratin powder used in this experiment was from wool. Keratin from the stratum corneum is difficult to obtain in a large amount, so it is not easy to make large tablets as employed in this experiment. Wool keratin is readily available and is more similar to the stratum corneum keratin than feather keratin on the basis of the X-ray diffraction patterns of the keratins.[24] Such keratin tablets are useful for evaluation of the moisturizing effects of several substances, and are especially useful as a model of the stratum corneum keratin. However, skin lipids such as cholesterol, cholesterol esters and phospholipids also have a moisturizing effect,[25] and keratin tablets are probably not useful to evaluate the skin-moisturizing effect on the lipid in the stratum corneum.

The *in vivo* method as shown in Fig. 8 should cover the moisturizing effects arising from both keratin and lipid in the stratum corneum. Pyrrolidones show highly hygroscopicity and water-holding capacity both *in vitro* and *in vivo* (Figs. 7 and 8). These results suggest that

pyrrolidones do affect the stratum corneum keratin. However, it is not yet clear whether pyrrolidones affect the stratum corneum lipid. Among the pyrrolidones, MP was a superior enhancer, since it showed the highest effect both *in vitro* and *in vivo*.

On the other hand, salicylates have no effect on the hygroscopicity and water-holding capacity in the stratum corneum. Salicylates rather affect the softening and dissolving capacities of the stratum corneum, as suggested from Fig. 9. SA shows the greater effect on the dissolution of keratin powder. However, the mechanisms of the enhanced permeations of IND induced by salicylates (SA) and pyrrolidones (MP) may be very different. Pyrrolidones may affect not only the keratin dissolution but also the water-holding capacity of the skin. The large lag time seen in SA treatment in Fig. 6 may be due to the gradual dissolution of keratin (Fig. 9). As the structural formulas of pyrrolidones are similar to that of Azone, the mechanism of action of these compounds may be similar.

If several enhancers which have different mechanisms of action were used simultaneously, a synergism might be obtained. In the present study, the effects of three combinations of two enhancers were measured. However, a useful result was not obtained (Fig. 10), though in the case of combined SA and GS, the permeation of IND up to 24 h was lower than that with SA alone and the permeation from 24 to 32 h was higher than that with GS alone. Further experiments might be worthwhile.

Irritancies and toxicities of enhancers were not considered in the present experiments. Research to test the safety of enhancers and also to establish the mechanism of action is necessary to develop useful penetration enhancers for transdermal drug delivery.

In conclusion, most enhancers which affected the skin permeation of IND in the present study probably acted on the stratum corneum. Although the mechanism of action has not been established in detail, pyrrolidones and salicylates do act on the keratin in the stratum corneum. Pyrrolidones such as MP have water-holding capacity in the stratum corneum and salicylates such as MS have a keratin-dissolving ability. Pyrrolidones may also act on the lipid, as do Azone and IMP. Such effects on the skin barrier result in changes of several parameters such as the partition coefficient (skin/vehicle) of IND and the diffusivity and activity coefficient of IND in the skin barrier.

Acknowledgement The authors thank Dr. Shuji Sato, Lion Corporation, for valuable discussions.

References and Notes

1) Part of this work was presented at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1986.
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