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Effect and mode of action of aliphatic esters on the in vitro skin permeation of nicorandil

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Summary

The effect and mode of action of aliphatic esters such as isopropyl myristate (IPM), which were known as percutaneous absorption enhancers, on the in vitro permeation of a drug across the excised hairless rat skin were investigated. Nicorandil and propylene glycol (PG) or water were used as a model drug and solvent in a drug-donor compartment. It was found that IPM markedly enhanced nicorandil permeation across the full-thickness skin, especially when PG was used as a solvent. The skin permeation profiles, e.g., flux and lag time, of nicorandil widely differed with the change of IPM content in PG. The large differences in flux and lag time were also affected by the alteration of kinds of aliphatic esters. That is, the flux increased inversely proportional to a lipophilic index of aliphatic esters derived from partition coefficient between *n*-hexane and water, and the lag time period was almost proportional to the lipophilic index. Fluxes of an IPM-PG treatment and PG treatment across the stratum corneum-stripped skin, however, were almost the same. The permeation-enhancing effect of aliphatic esters could not be explained only by the effects of the aliphatic esters on the solubility of nicorandil in vehicle and the release of the drug from vehicle. On the other hand, the skin permeation profiles of solvents (PG and water) were almost the same as the nicorandil permeation. Therefore, aliphatic esters such as IPM would mainly act on the stratum corneum, and would increase the diffusivity in the stratum corneum and/or partition coefficient between the stratum corneum and vehicle both of the drug and solvent.

Introduction

The skin has been recognized as a new site for administration of systemically active drugs. However, the skin permeability of drugs is generally poor. Therefore, various methods for enhancing the skin permeability of drugs have been examined. The method using percutaneous absorption enhancers, e.g., dimethylsulfoxide (Barry, 1983), pyrrolidones (Southwell et al., 1981) and Azone (Stoughton and McClure, 1983, Sugibayashi et al., 1985), is common, and a number of reports have so far been published. It is also known that aliphatic esters such as isopropyl myristate (IPM) have additive percutaneous absorption-enhancing effects (Washitake et al., 1981), although the mechanism of action is still obscure.

In the present study, we have used nicorandil (Fig. 1), a potent coronary vasodilator (Nakagawa et al., 1979), as a model drug to attain a better

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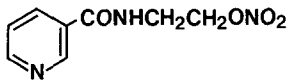

Fig. 1. Chemical structure of nicorandil.

understanding of the mode of the percutaneous absorption-enhancing action of aliphatic esters. An excised hairless rat skin was used in all experiments.

Materials and Methods

Materials

Nicorandil, N-(2-hydroxyethyl) nicotinamide nitrate, was supplied by Nisshin Flour Milling Co. (Tokyo, Japan). IPM, isopropyl *n*-butyrate (IPB), isopropyl *n*-hexanoate (IPH), isopropyl *n*-decanoate (IPD) and isopropyl palmitate (IPP) were purchased from Tokyo Chemical Industry Co. (Tokyo). Propylene glycol (PG) and deuterium oxide (D_2O) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Octyldodecyl myristate (ODM) was kindly supplied by Kao Corporation (Tokyo). All other chemicals and solvents were reagent grade and obtained commercially.

Animals

Male hairless rats (WBN/kob strain), with an approximate weight of 150 g each, were supplied by Saitama Laboratory Animals (Saitama, Japan).

Skin membrane preparation

The abdominal region of the hairless rats was carefully shaved. A 2-cm^2 section of the left and right abdominal skin was excised and mounted between two half diffusion cells, each having 2.0 ml volume and 0.636 cm^2 effective diffusion area (Morimoto et al., 1986). Stripped skin was obtained by 20 times stripping of the stratum corneum from the shaved abdominal skin with adhesive cellophane tape (Nichiban Co., Tokyo) (Washitake et al., 1973).

Permeation procedure

The dermis side of the skin was in contact with the receiver compartment and either the stratum corneum (full-thickness skin) or epidermis (stripped skin) with the donor compartment. The receiver compartment of each cell was filled with 2 ml of physiological saline and the donor compartment with 2 ml of drug suspension in each solvent. In the solvent permeation studies, the donor compartment was filled with 2 ml of drug-free solvent. The diffusion cells were maintained at 37 °C in a water bath. The donor and receiver compartments were mixed throughout the experiment with a Teflon stirrer driven by a 150 rpm constant speed motor. At appropriate times, 100-μl samples were withdrawn from the receiver compartment, and 100 μl internal standard solutions (1 μg/ml of ethyl *p*-hydroxybenzoate methanol solution) were added to them. After sampling, 100 μl of saline were added to the receiver compartment to keep the volume constant.

Solubility determination

The solubilities of nicorandil in water, PG, aliphatic esters and aliphatic ester–PG mixtures were determined by the following method. Excess drug was added to 5 ml of test solvent, and the suspension was stirred in a water bath at 37 °C. After equilibrium, a part of the solution was withdrawn and filtered quickly through a membrane filter (Type RC, Toyo Roshi, Tokyo). The filtrates were diluted with methanol or isopropanol, and subsequently determined for nicorandil content using an HPLC.

Release procedure

The diffusion cell as above was employed for determination of the release rates of nicorandil from aliphatic ester–PG mixed vehicles. A dialysis membrane (Union Carbide Corp., U.S.A.) was mounted between two half cells, and the donor compartment was filled with 2 ml of 0.5% nicorandil solution and the receiver compartment with 2 ml of water. The diffusion cells were maintained at 37 °C in a water bath. The donor and receiver compartments were mixed throughout the experiment. Samples were withdrawn from the receiver compartment for assay at appropriate times over 6 h period.

Analysis

Concentrations of nicorandil and PG in the receiver phase were determined using an HPLC and a GC-FID, respectively.

HPLC conditions were as follows: pump, LC-6A (Shimadzu, Kyoto, Japan); column, 4.6 mm × 250 mm stainless-steel column packed with Nucleosil $5C_{18}$ (Macherey Nagel, Germany); mobile phase, water : acetonitrile (6 : 4); detector, UV 254 nm.

GC-FID conditions were as follows: instrument, GC-6A (Shimadzu); column, 3 mm × 1 m glass column packed with Gaschropak-54 (Gaskuro Kogyo, Tokyo); column temp., 200 °C; N_2 gas flow rate, 40 ml/min.

Determination of D_2O in the receiver phase was carried out by measuring the intensity of the 2512 cm^{-1} deuterium oxide band in the infrared absorption spectrum of a sample diluted with water (Thornton and Condon, 1950). The absorbance of the diluted sample in a calcium fluoride cell was determined with a Shimadzu model IR-450 infrared spectrometer.

Partition coefficient of aliphatic esters

Equivalent volumes (10 ml each) of *n*-hexane and water was added to 100 mg of each aliphatic ester in a glass-stoppered tube. After equilibrium by vigorous stirring of the mixture for 30 min at 25 °C, the *n*-hexane layer was transferred to another test tube. The concentration of aliphatic ester in the *n*-hexane was determined by a GC-FID equipped with 3 mm × 1 m glass columns packed with 3% OV-17. The partition coefficient of aliphatic ester, P, between *n*-hexane and water was calculated as follows:

$$P = \frac{C_{hex}}{C_{tot} - C_{hex}}$$

where C_{tot} and C_{hex} are initial and equilibrium concentrations of aliphatic esters in *n*-hexane.

The logarithm of the partition coefficient, log P, was employed as a lipophilic index of aliphatic esters.

Results

Effect of IPM on the permeation of nicorandil across the full-thickness skin

The effect of IPM on the permeation of nicorandil across the full-thickness skin was investigated. Fig. 2 shows the time course of the cumulative amount of nicorandil permeated per unit area from PG or water suspension with or without IPM. Content of IPM was 10% (w/w) each. IPM significantly enhanced the nicorandil permeation, especially in PG solvent, and steady-state flux (flux) of an IPG-PG treatment was ca. 70 times higher than that of a PG treatment (IPM-free). Further experiments were mainly carried out in IPM-PG mixed solvent.

Effect of IPM on the permeation of nicorandil across the stripped skin

As the main barrier for the drug permeation is the stratum corneum of the skin, the effect of IPM on the permeation of nicorandil across the stratum corneum-stripped skin was investigated as shown in Fig. 3. In the IPM-PG treatment, the nicorandil permeation across the stripped skin was almost the same as that across the full-thickness skin. In the PG treatment, on the contrary, the nicorandil

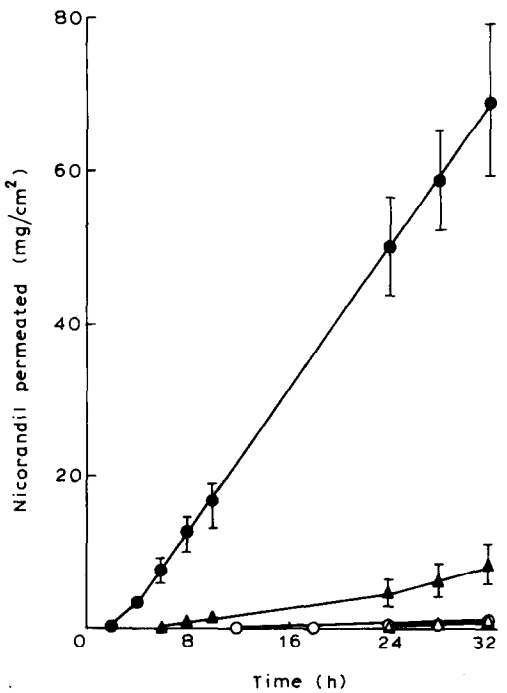

Fig. 2. Effect of IPM on the permeation of nicorandil across the full-thickness skin. ○, PG; ●, PG with 10% IPM; △, water; ▲, water with 10% IPM. Each point represents the mean ± S.E.M. of 3–5 experiments.

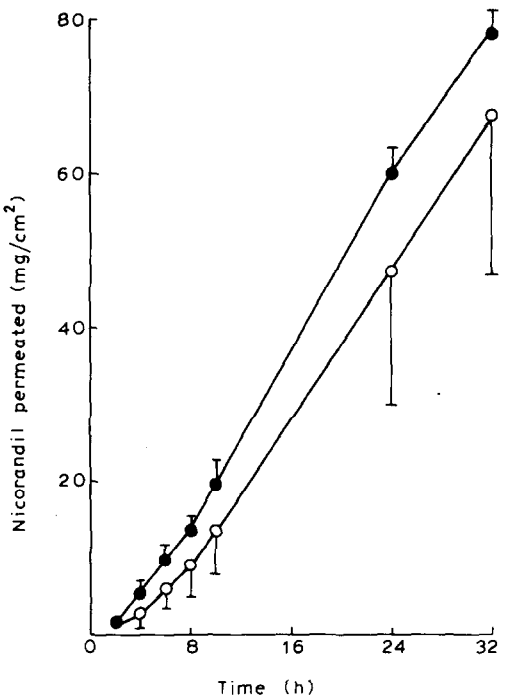

Fig. 3. Effect of IPM on the permeation of nicorandil across the stripped skin. ○, PG; ●, PG with 10% IPM. Each point represents the mean ± S.E.M. of 3 experiments.

permeation across the stripped skin was much higher than that across the full-thickness skin. In conclusion, the flux across the full-thickness skin with IPM was comparable to both fluxes with IPM and without IPM across the stripped skin.

Relationship between IPM content and nicorandil permeation

The effect of IPM content (0–100% (w/w) in PG) on the nicorandil permeation across the full-thickness skin was investigated. The flux and time taken to reach a steady-state level (lag time) were employed as indications for the skin permeation of nicorandil. Fig. 4 shows the relationships between IPM content and the flux, and between IPM content and the lag time, respectively. The flux was markedly increased by addition of 1% IPM compared to that without IPM, and was kept approximately constant up to 50% IPM. On the other hand, the lag time between the PG treatment and 1% IPM-PG treatment did not show a marked difference. The lag times in the IPM-PG treatments containing more than 5% IPM were significantly shorter than those in the PG treatment and 1% IPM-PG treatment. The addition of IPM to PG would contribute not only to an increase in the flux but also to a shortening of the lag time in the skin permeation of nicorandil.

Relationship between lipophilicity of aliphatic esters and nicorandil permeation

In order to make the effect of IPM clear, the nicorandil permeations were measured with other aliphatic esters. Chemical structures of aliphatic esters and their lipophilic indexes, log P, which were derived from the partition coefficient between *n*-hexane and water, were shown in Table 1. The lipophilic index, log *P*, proportionally increased with the molecular weight of aliphatic esters. The content of aliphatic ester in PG was 10% (w/w) in the permeation experiment. Fig. 5 shows the relationships between the log *P* and flux, and between the log *P* and lag time. Large differences in flux and lag time were found by changing the kinds of aliphatic esters, that is, the flux increased inversely proportional to the log *P* and the lag time was almost proportional to the log *P*. But the flux and lag time in an IPB treatment were almost the same as that in an IPH treatment despite of the difference in lipophilicity.

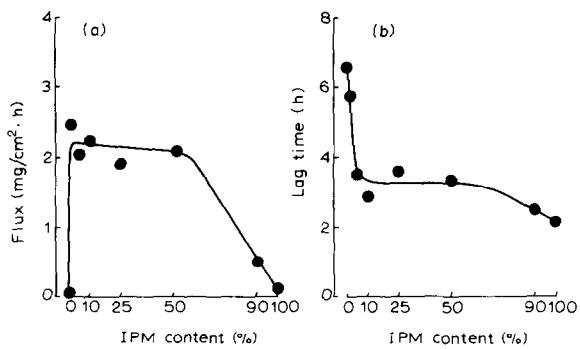

Fig. 4. Effect of IPM content on the permeation of nicorandil in PG. a: relationship between IPM content and flux. b: relationship between IPM content and lag time. Each point represents the mean of 2–4 experiments.

TABLE 1

Chemical structures and lipophilic indexes, log P, of aliphatic esters (R_1COOR_2)

Subject	MW	R_1	R_2	log P
Isopropyl *n*-butyrate (IPB)	130	$CH_3(CH_2)_2-$	$-CH(CH_3)_2$	1.12
Isopropyl *n*-hexanoate (IPH)	158	$CH_3(CH_2)_4-$	$-CH(CH_3)_2$	1.38
Isopropyl *n*-decanoate (IPD)	214	$CH_3(CH_2)_8-$	$-CH(CH_3)_2$	1.69
Isopropyl myristate (IPM)	270	$CH_3(CH_2)_{12}-$	$-CH(CH_3)_2$	1.96
Isopropyl palmitate (IPP)	298	$CH_3(CH_2)_{14}-$	$-CH(CH_3)_2$	2.09
Octyldodecyl myristate (ODM)	508	$CH_3(CH_2)_{12}-$	$-CH_2CH(C_{10}H_{21})$	2.79

MW, molecular weight; P, partition coefficient between *n*-hexane and water at 25 °C.

Effect of aliphatic esters on the solubility and release of nicorandil

Fig. 6 shows the percent releasing of nicorandil from each IPM-PG mixed vehicle as a function of time. The release rates were enhanced by increasing the IPM content. Fig. 7 shows the percent releasing of nicorandil over 4 h and solubilities of the drug in IPM-PG mixtures against the IPM content. Since IPM is immiscible with PG, the solubilities of nicorandil decrease linearly by addition of IPM. By Higuchi's theory (Higuchi, 1960), the skin permeation flux of a drug is proportional to the product of solubility in vehicle and partition coefficient between vehicle and skin barrier. In the present study, it is assumed that the percent release of nicorandil from vehicle to water reflects upon the partition coefficient of the drug between vehicle and skin barrier. The products of the solubility, C_s, and the percent releasing of the drug, R(%), over 2, 4 or 6 h were calculated and illustrated in Fig. 8. Although the profiles as shown in Fig. 8 were similar to that in Fig. 4a, the behaviors in the PG treatment between Fig. 4a and Fig. 8 were entirely different. Therefore, the enhancing effect of IPM-PG mixture on the permeation of nicorandil (Fig. 4a), especially at low content of IPM, could not be explained only by the change of the solubility of the drug in vehicle and the release of the drug from vehicle.

In a similar manner, the solubilities of nicorandil in aliphatic esters and in 10% aliphatic ester-PG mixtures were determined and illustrated

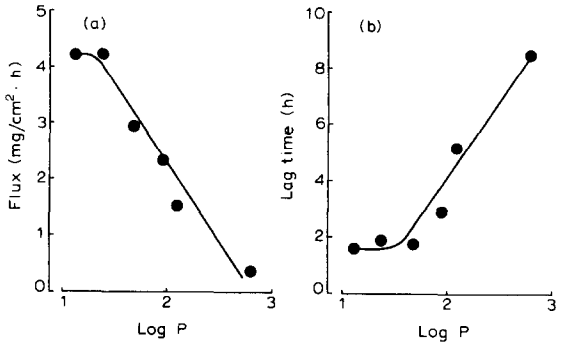

Fig. 5. Effect of lipophilicity of aliphatic esters on the permeation of nicorandil in PG. a: Relationship between log P and flux. b: Relationship between log P and lag time. Each point represents the mean of 2–3 experiments.

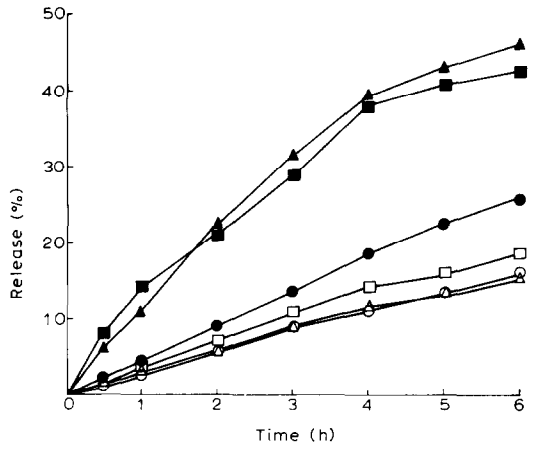

Fig. 6. Effect of IPM content on the release of nicorandil from IPM-PG mixed vehicles. IPM content in PG (%): ○, 0%; △, 10%; □, 25%; ●, 50%; ▲, 90%; ■, 100%. Each point represents the mean of two experiments.

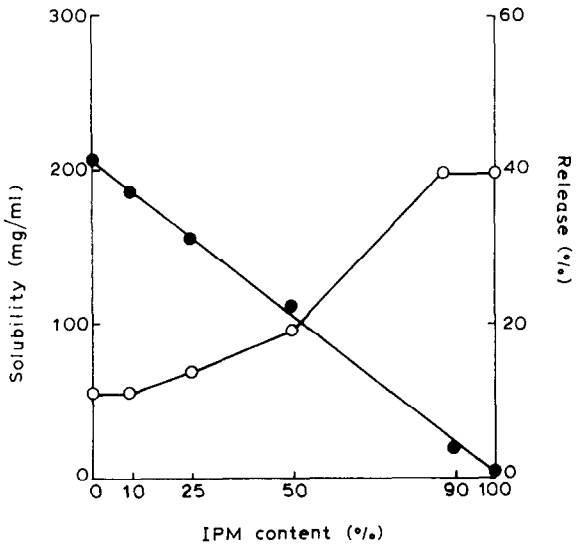

Fig. 7. Effect of IPM content on the solubility (●) of nicorandil and percent releasing of the drug over 4 h (○).

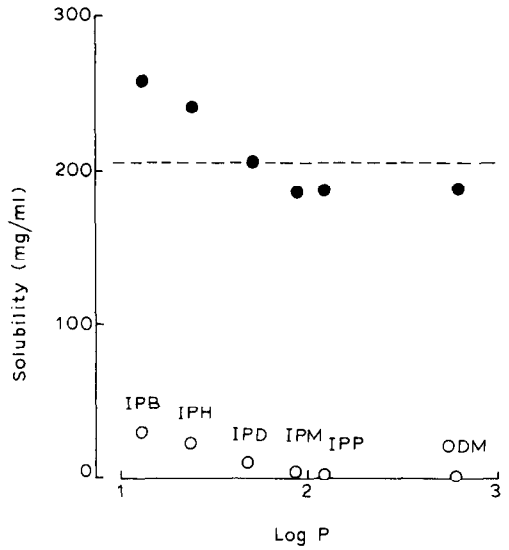

Fig. 9. Solubility of nicorandil in aliphatic esters (○) and in 10% aliphatic ester-PG mixtures (●). The dotted line represents the solubility in PG.

in Fig. 9. In the figure, log P was employed as abscissa in order to compare with Fig. 5. Since the solubility of nicorandil in IPB or IPH was greater than those in other aliphatic esters and IPB or IPH was miscible with PG, the solubility of nicorandil in 10% IPB-PG or 10% IPH-PG mixtures was higher than that in PG. However, the solubility in IPB-PG mixture was increased only about 30% compared to that in PG, and the release rate of nicorandil from IPB-PG mixture was similar to that from IPM-PG or ODM-PG mixture (Fig. 10). Therefore, the increase in flux by aliphatic esters (Fig. 5a) could also not be explained only by the solubility and drug release.

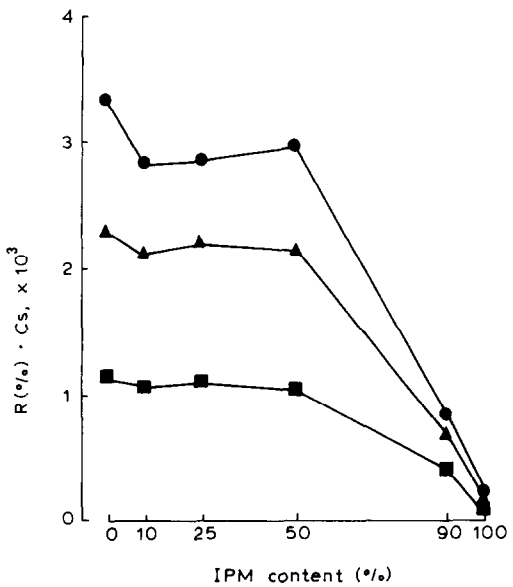

Fig. 8. Effect of IPM content on the $R(\%)\cdot C_s$. ●, $R(\%)$ over 6 h; ▲, $R(\%)$ over 4 h; ■, $R(\%)$ over 2 h.

Effect of aliphatic esters on the permeation of solvent across the full-thickness skin

The permeation of solvent (PG) in the donor compartment was investigated in the case of treatment with or without aliphatic esters. The time courses of the cumulative amount of PG permeated per unit area were indicated in Fig. 11 when IPM content was varied in the range of 0–90% (w/w), and in Fig. 12 when the kind of aliphatic esters was varied. The permeation of PG was similar to the corresponding nicorandil permeation in all treatments. On the other hand, the amount of another constituent, IPM, permeated from the donor compartment in 50% IPM-PG treatment was below 1 mg/cm^2 at 32 h.

In Fig. 13a, the amount of nicorandil permeated at each sampling time was plotted against

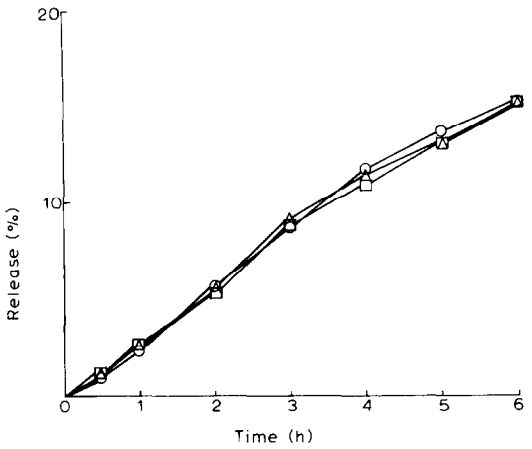

Fig. 10. Effect of aliphatic esters on the release of nicorandil from aliphatic ester-PG mixed vehicles. ○, IPB; △, IPM; □, ODM (10% each). Each point represents the mean of 2 experiments.

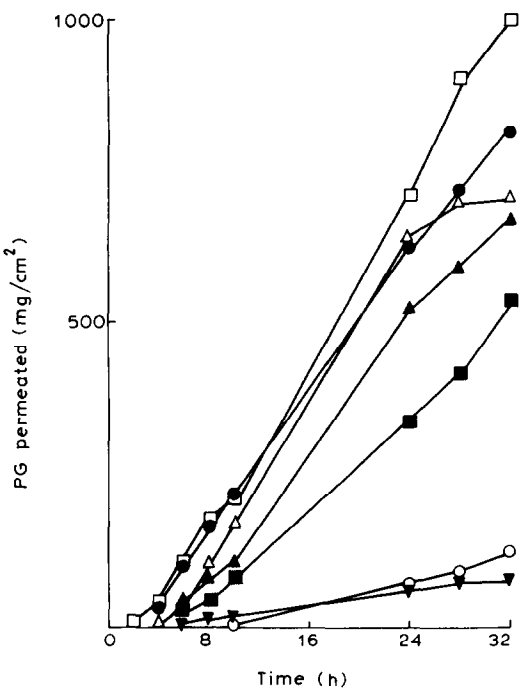

Fig. 11. Effect of IPM content on the permeation of PG across the full-thickness skin. IPM content in PG (%): ○, 0%; △, 1%; □, 5%; ●, 10%; ▲, 25%; ■, 50%; ▼ 90%. Each point represents the mean of 2 experiments.

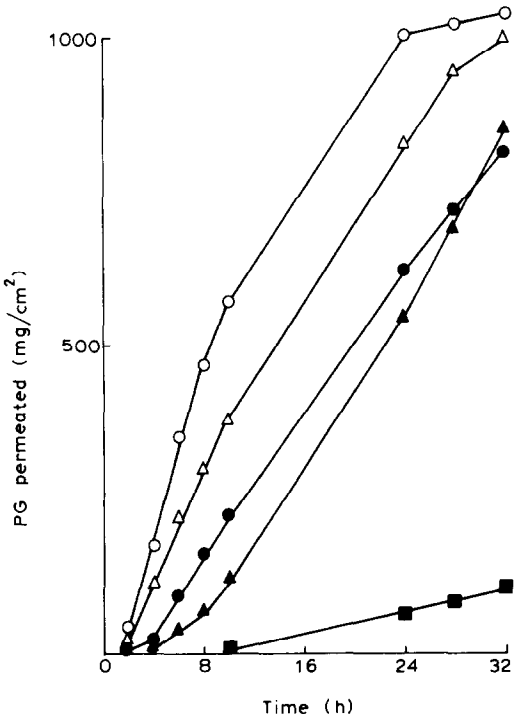

Fig. 12. Effect of aliphatic esters on the permeation of PG across the full-thickness skin. Key: ○, IPH; △, IPD; ●, IPM; ▲, IPP; ■, ODM (10% each). Each point represents the mean of 2 experiments.

the amount of PG permeated at the corresponding time. The amounts of nicorandil permeated were increased in proportion to the amount of PG permeated in both treatments with 10% IPM and without IPM. For the purpose of making the relationship between the drug and PG permeation clear, a tentative apparent amount of nicorandil permeated, Q_{cal} (mg/cm^2), was calculated from the amount of PG permeated, Q_{slv} (mg/cm^2), and solubility of nicorandil at 37 °C, C_s (g/cm^3). Q_{cal} was calculated as follows:

$$Q_{cal} = Q_{slv} \cdot C_s/\rho$$

where ρ (g/cm^3) is the density of the solvent at 25 °C. The observed amount of nicorandil permeated, Q_{obs} (mg/cm^2), was plotted against Q_{cal} as shown in Fig. 13b. Apparent correlations were present between the skin permeations of nicorandil and those of PG, since a linear relationship

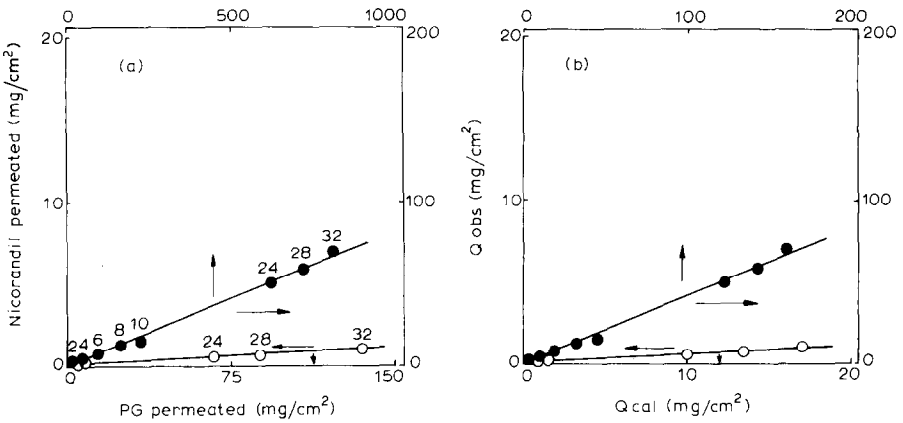

Fig. 13. Relationship between skin permeations of nicorandil and PG. a: nicorandil permeation * vs. PG permeation. b: observed (Q_{obs}) * vs. calculated (Q_{cal}) amounts of nicorandil permeation. ○, PG; ●, PG with 10% IPM. The number on each symbol indicates the measuring time (h). * These data are the same.

existed between Q_{obs} and Q_{cal} in both treatments with and without IPM.

Similarly the permeation of water was investigated with 10% IPM and without IPM. Water permeation was estimated by using D_2O, because water (H_2O) was present abundantly in the skin and receiver compartment. The flux of D_2O was increased by addition of IPM. The increasing ratio of D_2O flux with/without IPM was smaller than that of PG flux with/without IPM as shown in Fig. 11. This profile was almost the same as the result of nicorandil permeation in Fig. 2. Q_{cal} was calculated in a similar manner as above, and compared with Q_{obs} as shown in Fig. 14. C_s was 0.0267g/cm^3 in water. The positive correlation was also found between the skin permeation of nicorandil and that of D_2O.

The gradient of the straight line, slope, and the correlation coefficient, r, were calculated from each value of Q_{obs} and Q_{cal} by the least-squares method as shown in Table 2. The correlation

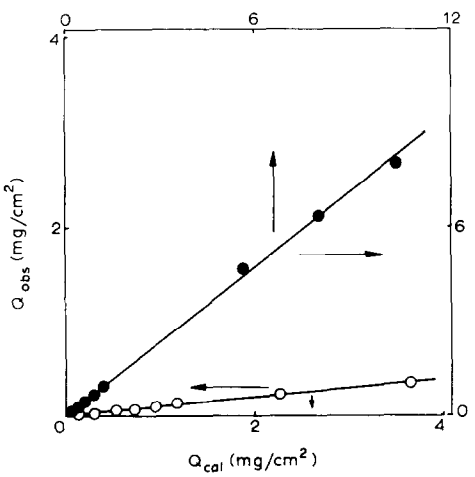

Fig. 14. Relationship between the calculated (Q_{cal}) and observed (Q_{obs}) amounts of the skin permeation of nicorandil in water. ○, water; ●, water with 10% IPM.

TABLE 2

Relationship between the calculated and observed amounts of the permeation of nicorandil

Solvent	Additive (%, w/w)		Slope	r
PG	None		0.057	0.998
	IPM	(1)	0.240	0.996
		(5)	0.235	0.996
		(10)	0.421	0.999
		(25)	0.407	0.996
		(50)	0.610	0.998
		(90)	0.811	0.998
	IPH	(10)	0.310	0.992
	IPD	(10)	0.342	0.999
	IPP	(10)	0.279	0.999
	ODM	(10)	0.419	0.998
Water	None		0.103	0.999
	IPM	(10)	0.789	0.997

Slope is obtained from a plot of Q_{obs} vs. Q_{cal}; r, correlation coefficient

coefficients were over 0.99 in all treatments, and the slopes had a tendency to approach unity by addition of IPM or other aliphatic esters to PG or water.

Discussion

It is well known that the skin permeability of a drug is proportional to the thermodynamic activity of the drug in vehicle, and that the thermodynamic activity of the drug is constant in a state of suspension independent of the vehicle (Higuchi, 1960). Under certain assumptions, e.g., vehicle does not affect the skin barrier and the receiver phase is kept in sink condition throughout the experiment, permeation flux of a drug would be constant in theory because the drug suspends in any vehicle. Therefore, the conversion to another chemical structure, such as a pro-drug, would be required in order to enhance the drug permeation across the skin. Actually, many studies have been approached from this side (Yu et al., 1979, Møllgaard et al., 1982). On the other hand, it has also been recognized that the skin permeations of drugs were increased by the use of percutaneous absorption enhancers such as Azone.

In the present paper, the effect and mode of action of aliphatic esters, which were found to be one of the absorption enhancers, on the in vitro skin permeation of nicorandil were investigated. All experiments were done in a state of nicorandil suspension in order to keep constant the thermodynamic activity of the drug in donor compartment. In the 10% IPM-PG treatment, the nicorandil permeation across the full-thickness skin was enhanced about 70-fold compared with the PG treatment, and the permeation across the stripped skin was approximately the same in the treatments with and without IPM. Therefore, it was suggested that the barrier property of the stratum corneum would be weakened by addition of IPM, and hence diffusivity of the drug would be increased and/or activity coefficient of the drug in skin barrier (stratum corneum) would be decreased. The above presumption would be supported as follows: that is, the permeation of nicorandil widely differed without being significantly affected by the solubility of the drug in vehicle and release of the drug from vehicle.

The skin permeations of the solvents such as PG and water (D_2O) from the donor compartment were measured and compared with the nicorandil permeation. A comparison was carried out by plotting Q_{obs} toward Q_{cal}. Consequently, it was found that a correlation was present between the skin permeations both of the solvent and drug. By considering the above results, the important role of solvents in vehicle on the skin permeation of a drug would be not only for the solubility and/or release of the drug but also for the function of "solvent-drag" (Kedem and Katchalsky, 1958, Karino et al., 1982). As the slope approached unity by addition of aliphatic esters (Figs. 13b and 14 and Table 2), the contributions of solvent drag to the skin permeation of nicorandil would be greater than the treatment without aliphatic esters. With respect to the direct action of IPM toward the stratum corneum, it was found in our laboratory that the permeation of IPM into the membrane of liposomal bilayers, which were regarded as a model of the stratum corneum lipid, made these membranes more fluid by measuring the change in fluorescence polarization using 1,6-diphenylhexatriene entrapped in the liposomes.

In conclusion, aliphatic esters would mainly act on the lipid in the stratum corneum, which is a main barrier against the drug permeation, and would increase the diffusivity in the stratum corneum and/or partition coefficient between the stratum corneum and vehicle of both the drug and solvent. Further studies would be required to clarify the relationship between the skin permeabilities of drugs and solvents.

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