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Enhancing effect of pyrrolidone derivatives on transdermal drug delivery. I.

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Summary

In order to develop potential dermal absorption enhancers, we prepared 9 pyrrolidone derivatives and compared their physicochemical characteristics, transdermal absorbabilities and promoting actions on transdermal delivery of phenolsulfonphthalein (Phenol red) as a model of unabsorbable drug. The preparing pyrrolidones included 1-methyl, 1-hexyl and 1-lauryl-2-pyrrolidone, and their 4-carboxy or 4-methoxycarbonyl substituted derivatives. They showed various lipophilicities according to the nature of an introduced functional group. In an in vitro experiment, phenol red applied with any pyrrolidone showed a penetration through an excised rat skin although the dye applied alone could not transfer. Especially 1-hexyl- and 1-lauryl- substituted derivatives showed superior promoting effect to 1-methyl-substituted derivatives. A lag time for Phenol red appearance to the receptor phase was prolonged with the elongation of a 1-substituted alkyl chain of enhancer. On the other hand, 1-methyl- and 1-hexyl-substituted derivatives penetrated through a rat skin but 1-lauryl-substituted derivatives showed little transfer. Introduction of a 4-carboxy or 4-methoxycarbonyl group to 1-alkyl-2-pyrrolidone reduced the enhancer permeability, decreased the promoting effect and prolonged the lag time. The hydrolysis of 4-methoxycarbonyl derivatives to 4-carboxy derivatives in the skin was observed. The skin accumulation of Phenol red at 10 h increased with an enhancement of dye transfer. However, there is no relationship between enhancer accumulation in the skin and its penetration. The promoting action of 1-methyl, 1-hexyl and 1-lauryl-2-pyrrolidone was supported by an in vivo absorption experiment.

Introduction

Recently, percutaneous drug delivery has attracted a great interest not only in local chemotherapy but also in systemic chemotherapy using controlled-release technology. If successful, this method of delivery should avoid first-pass effects, maintain constant plasma levels of drugs and reduce problems of patient compliance (Shaw, 1982; Chien, 1983). However, efforts in this direction have been limited by the drawback that most drugs will not penetrate at rates sufficiently high for therapeutic efficacy.

Numerous attempts have been reported to improve the absorbability by pharmaceutical approaches. One promising approach to enhancement of percutaneous absorption is to develop

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prodrugs which revert to the active compound by virtue of enzymatic or chemical lability in the skin (Yu et al., 1980; Møllgaard et al., 1982; Mukai et al., 1985). Another approach is the use of a transdermal absorption enhancer, which seems to be advantageous to transdermal application for most drugs. Many compounds have been evaluated in the hope of obtaining compounds with improved transdermal delivery. However, most of them have not become available for clinical use because of their side effect as excipient. In addition, there has been little systematic study about chemical approach for designing the useful enhancer.

The physicochemical property and percutaneous absorption behavior of enhancer can be considered as one of the most important factors controlling the pattern of enhancing effects and side effects. On the basis of this consideration, in the present study, 1-methyl-2-pyrrolidone reported as a potential enhancer was derivatized to various lipophilic or hydrophilic forms and their absorption behavior and promoting action on transdermal penetration of phenolsulfonphthalein (Phenol red) as a model of unabsorbable drug were investigated. Transdermal absorption of Phenol red was evaluated by in vitro and in vivo techniques using rats as a model animal.

Materials and Methods

Chemicals

1-Methyl-2-pyrrolidone(**I**) was gained commercially (Nakarai Chemicals, Ltd., Kyoto, Japan) and used without further purification. The other pyrrolidone derivatives were prepared by a modified usual method (Zienty and Steahly, 1947; Paytash et al., 1950). NMR spectra were taken on a Hitachi R-600 FT-NMR spectrometer (Hitachi Co., Ltd., Tokyo, Japan) and elemental analyses were performed by the Center for Organic Elemental Microanalysis, Nagasaki University. The analytical results obtained were within $\pm 0.4\%$ of the theoretical values. Thin-layer chromatographic (TLC) analysis of pyrrolidones was performed on silicagel plates (60F254, Merck). A solvent system of chloroform–ethyl acetate–acetic acid–methanol (90 : 7 : 3 : 10 v/v/v/v) was used. The products on TLC plate were detected by staining with Bromocresol green and iodine after removing the solvent completely. Phenol red and all other reagents were of reagent grade.

Synthesis

1-Alkyl-2-pyrrolidone. To 300 ml of γ-butyrolactone, an equimolar quantity of hexylamine was added and refluxed at 180 °C for 7 days. The end of reaction was confirmed by TLC, and the mixture was distilled to give a colorless fluid, 1-hexyl-2-pyrrolidone (**II**), at about 38% yield; bp 126–128 °C at 1.8 mmHg; ^{1}H-NMR(in $CDCl_3$) δ: 3.33(2H, t, $J = 9.0$ Hz, 5-H), 3.22(2H, t, $J = 9.0$ Hz, N-C$\underline{H_2}$-C_5H_{11}), 2.23(2H, t, $J = 8.0$ Hz, 3-H), 2.06(2H, t, $J = 9.0$ Hz, 4-H), 1.7-0.6(11H, m, N-CH_2-$C_5\underline{H_{11}}$ 1-Lauryl-2-pyrrolidone (**III**) was prepared in a similar manner; yield 35%; bp 194–196 °C at 0.5 mmHg; ^{1}H-NMR(in $CDCl_3$) δ: 2.90(2H, t, $J = 9.0$ Hz, 5-H), 2.78(2H, t, $J = 9.0$ Hz, N-C$\underline{H_2}$-$C_{11}H_{23}$), 1.80(2H, t, $J = 9.0$ Hz, 3-H), 1.62(2H, t, $J = 9.0$ Hz, 4-H), 1.20-0.20(23H, m, N-CH_2-$C_{11}\underline{H_{23}}$).

1-Alkyl-4-carboxy-2-pyrrolidone. To 300 g of itaconic acid, an equimolar quantity of methylamine was added and stirred at 150 °C for 4 h. The end of the reaction was confirmed by TLC, and the mixture was dissolved in acetone and filtrated. From the acetone solution, a white solid was recrystallized to get the precipitate of 1-methyl-4-carboxy-2-pyrrolidone(**IV**) in 83% yield; mp 151–153 °C; ^{1}H-NMR(in D_2O) δ: 3.73(2H, d, $J = 8.0$ Hz, 5-H), 3.56(1H, t, $J = 10.0$ Hz, 4-H), 2.83(3H, s, N-CH_3), 2.72(2H, d, $J = 9.0$ Hz, 3-H). Two other 4-carboxy-2-pyrrolidones were prepared in a similar manner. 1-Hexyl-4-carboxy-2-pyrrolidone(**V**) was recrystallized from hexane; mp 41–44 °C; ^{1}H-NMR(in $CDCl_3$) δ: 3.55(2H, d, $J = 9.0$ Hz, 5-H), 3.20(1H, t, $J = 9.0$ Hz, 4-H), 3.18(2H, t, $J = 9.0$ Hz, N-C$\underline{H_2}$-C_5H_{11}), 2.65(2H, d, $J = 10.0$ Hz, 3-H), 1.65-0.50(11H, m, N-CH_2-$C_5\underline{H_{11}}$).

1-Lauryl-4-carboxy-2-pyrrolidone(**VI**); mp 38–41 °C; ^{1}H-NMR(in $CDCl_3$) δ: 3.53(2H, d, $J = 10.0$ Hz, 5-H), 3.18(1H, t, $J = 9.0$ Hz, 4-H), 3.16(2H, t, $J = 9.0$ Hz, N-C$\underline{H_2}$-$C_{11}H_{23}$), 2.64(2H, d, $J = 9.0$ Hz, 3-H), 1.67-0.31(23H, m, N-CH_2-$C_{11}\underline{H_{23}}$).

1-Alkyl-4-methoxycarbonyl-2-pyrrolidone. The diazomethane was prepared as ether solution by reaction of *p*-nitrotoluene sulphonate with 50% potassium hydroxide. To the ether containing diazomethane, methanol solution of compound **IV** was added under excess diazomethane. The reaction mixture was left overnight with anhydrous sodium sulfate and filtrated. The filtrate was evaporated and the residual oily product was distilled under a reduced pressure to get 1-methyl-4-methoxycarbonyl-2-pyrrolidone(**VII**) as colorless fluid in about 35% yield; bp 141–144 °C at 0.5 mmHg; mp 13–17 °C; ^{1}H-NMR(in $CDCl_3$) δ: 3.72(3H, s, $COOCH_3$), 3.60(2H, d, $J = 10.0$ Hz, 5-H), 3.25(1H, t, $J = 10.0$ Hz, 4-H), 2.84(3H, s, N-CH_3), 2.66(2H, d, $J = 9.0$ Hz, 3-H). Two other 4-methoxycarbonyl derivatives were prepared in a similar manner.

1-Hexyl-4-methoxycarbonyl-2-pyrrolidone (**VIII**); bp 182–185 °C at 0.5 mmHg; ^{1}H-NMR(in $CDCl_3$) δ: 3.74(3H, s, $COOCH_3$), 3.61(2H, d, $J = 9.0$ Hz, 5-H), 3.30(1H, t, $J = 9.0$ Hz, 4-H), 3.20(2H, t, $J = 8.0$ Hz, N-CH_2-C_5H_{11}), 2.66(2H, d, $J = 10.0$ Hz, 3-H), 1.80-0.70(11H, m, N-CH_2-C_5H_{11}).

1-Lauryl-4-methoxycarbonyl-2-pyrrolidone (**IX**); bp 232–235 °C at 0.5 mmHg; mp 25–30 °C; ^{1}H-NMR(in $CDCl_3$) δ: 3.49(3H, s, $COOCH_3$), 3.36(2H, d, $J = 9.0$ Hz, 5-H), 3.04(1H, t, $J = 8.0$ Hz, 4-H), 3.02(2H, t, $J = 8.0$ Hz, N-CH_2-$C_{11}H_{23}$), 2.43(2H, d, $J = 10.0$ Hz, 3-H), 1.53-0.45(23H, m, N-CH_2-$C_{11}H_{23}$).

In vitro transfer experiment through rat skin

The in vitro diffusion cell was similar to the type used by Loftsson and Bodor (1981). The diffusion membranes were full-thickness abdominal skins of male Wistar albino rats weighing 250–300 g. The hair of the rat was removed with an animal clipper and a shaver at 24 h before the experiment. In preparing stripping skin, the abdominal part was stripped 15 times with scotch tape. The skin excised was mounted in the diffusion cell and the receptor phase was filled with isotonic sodium phosphate-buffered saline (pH 7.4, 49 ml) containing kanamycin sulfate (100 ppm). Test formulations were prepared by suspending Phenol red (200 mg) in isopropyl myristate (1 ml) containing pyrrolidone derivatives (2 mmol) or none. They were gently applied on the donor side of the skin surface which had an available diffusion area of 6.8 cm^2. The device, formulation and medium had been maintained at 32 °C at least for 10 h before the experiment. The diffusion cell was placed in a thermostated chamber maintained at 32 °C and the receptor phase was stirred by a magnetic stirrer covered with an adiabator. At appropriate intervals, samples of the receptor fluid were withdrawn for 10 h. At the end of the transfer period, the donor phase was washed with water and the excised skin was removed, homogenized and supplied for HPLC assay.

In vivo transfer experiment through rat skin

Male Wistar albino rats weighing 250–300 g who were shaven as in the in vitro experiment were used under anesthesia with pentobarbital, given i.p. A glass chamber (inside available diffusion area of 6.8 cm^2) was fixed on the surface of a rat abdomen with a cyanoacrylate adhesive (3000RS, Semedain Co., Ltd., Tokyo, Japan). After the same formulation as in an in vitro experiment was applied on the skin surface, 0.3 ml of blood and urine samples were carefully collected at fixed time intervals through a jugular vein and a polyethylene tubing cannulated into the bladder, respectively. Immediately the blood and urine samples were centrifuged at 1700 *g* for 3 min. The supernatants were used for measurement of phenol red content.

Determination of apparent partition coefficient and solubility

Apparent partition coefficients of compounds **I–IX** were determined in a chloroform or 1-octanol (10 ml) with distilled water (30 ml) system at 32 °C according to the method of Kakemi et al. (1967). The lipophilic indices (log k') were determined by HPLC employing the equation log $k' = \log[(t_r - t_0)/t_0]$, where t_r is the retention time and t_0 is the elution time of solvent. The log k' values were extrapolated to 0% methanol concentration to obtain the lipophilic indices (log k'_0).

The solubilities of compounds in *n*-hexane and distilled water were determined by suspending excess amounts of them in each of the solvents, followed by filtration and analysis.

Analysis

The pyrrolidone derivatives were determined by the use of HPLC system (LC-5A pump, SIL-1A injector, Shimadzu Co., Ltd., Kyoto, Japan) equipped with a variable wavelength UV absorbance detector (SPD-2A, Shimadzu Co., Ltd.) in a reverse phase mode. The stationary phase used was a Cosmosil $5C_{18}$ packed column (diameter 4.6 mm, length 150 mm, Nakarai Chemicals, Ltd.) and the peak was detected at 205 nm. The column was used at room temperature. Mixtures of methanol–water (I, 5 : 95; II, 55 : 45; III, 85 : 15; VII, 10 : 90; VIII, 55 : 45; IX, 82 : 18) or methanol–10 mM citrate buffer (IV, 1 : 99; V, 15 : 85; VI, 55 : 45) were used as the mobile phase at flow rate of 1.0 ml/min. The mobile phase was filtered by passing through a 0.45-μm pore size membrane filter (Toyo Roshi Co., Ltd., Tokyo, Japan). The standard solutions were chromatographed and calibration lines were constructed on the basis of peak-area measurements.

The Phenol red was assayed with a spectrophotometer (UV 110, Hitachi Co., Ltd.) at 550 nm under alkaline conditions by diluting with 1 M NaOH. The phenol red in a plasma sample was determined by an absorbance difference between 560 nm and 580 nm since the plasma interfered with the determination. The calibration curve showed a straight line.

Results

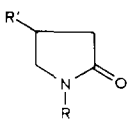
Chemistry

The pyrrolidone derivatives studied in the present paper included 1-methyl, 1-hexyl and 1-lauryl-2-pyrrolidone(**I–III**), and their 4-carboxy (**IV–VI**) or 4-methoxycarbonyl substituted derivatives(**VII–IX**). Their structures and physicochemical constants are summarized in Table 1.

The derivatives showed various lipophilicity, as expected, due to introduction of the functional group. The elongation of the alkyl side chain increased the lipophilicity of pyrrolidone and further introduction of carboxy group decreased it. They also exhibited various organic and aqueous solubilities. Most of the 4-carboxy and 4-methoxycarbonyl derivatives had higher melting point compared to the corresponding 1-alkyl-2-pyrrolidones(**I**, **II** and **III**).

TABLE 1

Structures and physicochemical properties of pyrrolidone derivatives



Compound	R	R′	MW	mp (°C)	log k_0' [a]	log P_0 [b]	log P_C [c]	Solubility at 32 °C (mM)	
								Water	Hexane
I	$-CH_3$	–H	99	< 4 [d]	0.56	−0.38	−1.0	m	578
II	$-C_6H_{13}$	–H	169	< 4 [d]	2.4	2.6	2.6	79.0	m
III	$-C_{12}H_{25}$	–H	253	< 4 [d]	5.1	4.2	4.2	0.032	m
IV	$-CH_3$	–COOH	143	151–153	−1.0	−1.5	−2.7	111	0.0003
V	$-C_6H_{13}$	–COOH	213	41–44	1.2	1.6	1.0	35.0	2.74
VI	$-C_{12}H_{25}$	–COOH	297	38–41	2.3	2.6	2.2	0.597	7.15
VII	$-CH_3$	$-COOCH_3$	157	13–17	0.80	−0.74	0.32	m	24.3
VIII	$-C_6H_{13}$	$-COOCH_3$	227	< 4 [d]	2.49	2.3	2.9	56.6	m
IX	$-C_{12}H_{25}$	$-COOCH_3$	311	25–30	5.1	4.0	4.3	0.042	m

[a] Lipophilic indices determined by HPLC (see text).
[b] Logarithmic value of apparent partition coefficient between 1-octanol and water.
[c] Logarithmic value of apparent partition coefficient between chloroform and water.
[d] The compound was liquid when stored at 4 °C.
[e] m, miscible.

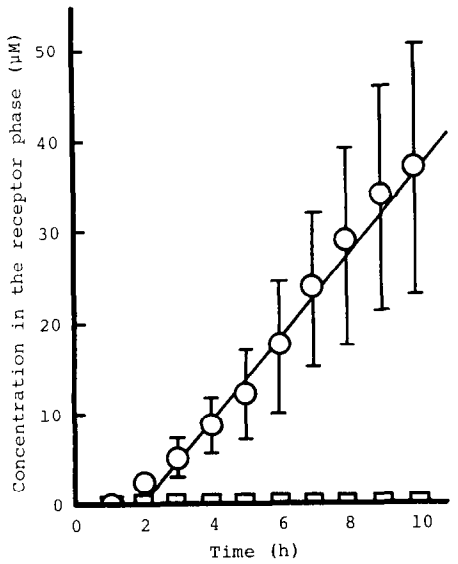


Fig. 1. Percutaneous penetration of Phenol red through an intact or stripping rat skin □; intact skin, ○; stripping skin. Vertical bars indicate S.E.M. and each point is the mean of 3 experiments.

In vitro transfer of Phenol red through rat skin

The transfers of Phenol red from isopropyl myristate through a full-thickness rat skin or a stripping skin are shown in Fig. 1. There was no detectable Phenol red in the receptor phase for 10 h through a rat skin. In contrast, the dye showed the high penetration through a stripping skin after a lag time of 2 h.

Effect of 1-alkyl-2-pyrrolidones on in vitro transfer of phenol red

The in vitro transfers of Phenol red and 1-alkyl-2-pyrrolidones (**I**, **II** and **III**) through a rat skin after their coapplication are shown in Fig. 2. Lipophilic compounds, II and III, largely promoted a transfer of phenol red (Fig. 2A) although **I** showed only a slight effect (insert in Fig. 2A). The lag time for Phenol red appearance in the receptor phase was observed as 0.7, 1.0 and 2.2 h for **I**, **II** and **III**, respectively. On the other hand, in the case of a transfer of enhancer itself, compound **I** transferred

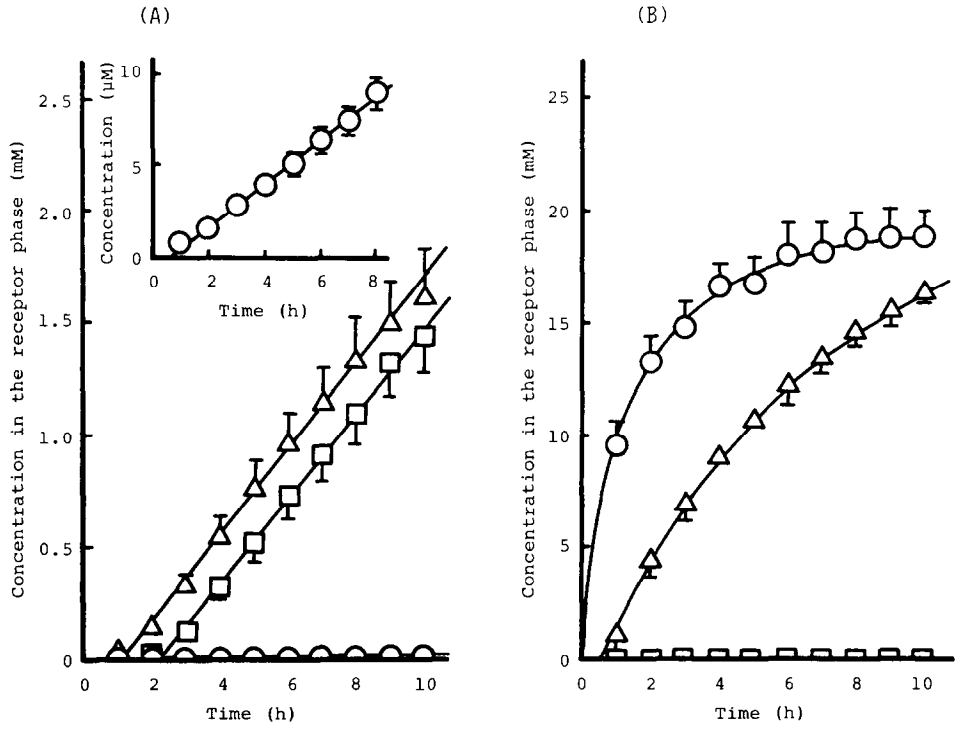


Fig. 2. Percutaneous penetration of Phenol red and 1-alkyl-2-pyrrolidones through a rat skin after their coapplication. A: Phenol red transfer. ○, **I**; △, **II**; □, **III**. Insert: an enlargement of the result of **I**. B: enhancer transfer. ○, **I**; △, **II**; □, **III**. Vertical bars indicate S.E.M. and each point is the mean of at least 6 experiments.

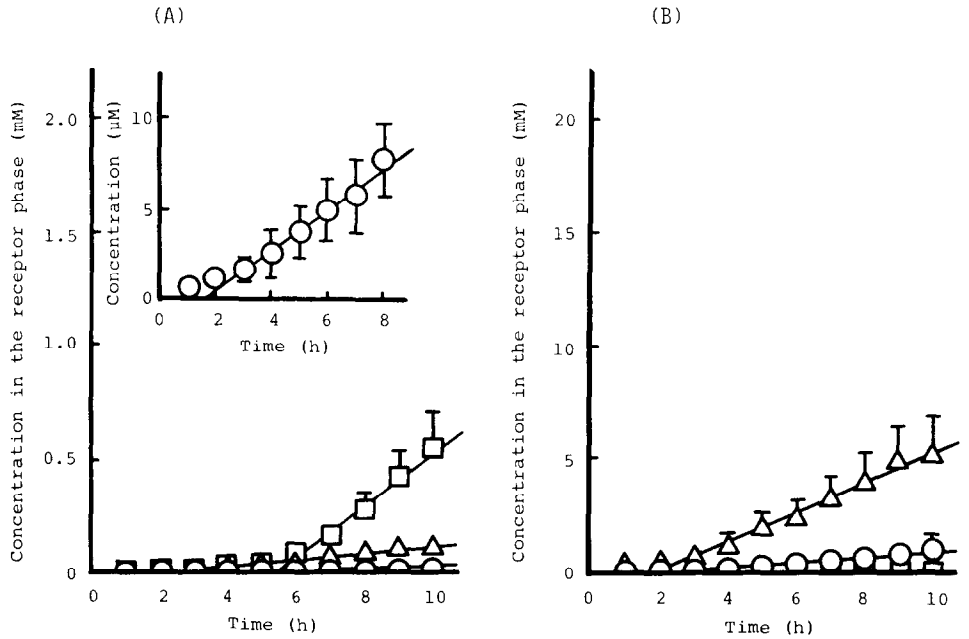


Fig. 3. Percutaneous penetration of Phenol red and 1-alkyl-4-carboxy-2-pyrrolidones through a rat skin after their coapplication. A: Phenol red transfer. ○, **IV**; △, **V**; □, **VI**. Insert: an enlargement of the result of **IV**. B: enhancer transfer. ○, **IV**; △, **V**; □, **VI**. Vertical bars indicate S.E.M. and each point is the mean of at least 6 experiments.

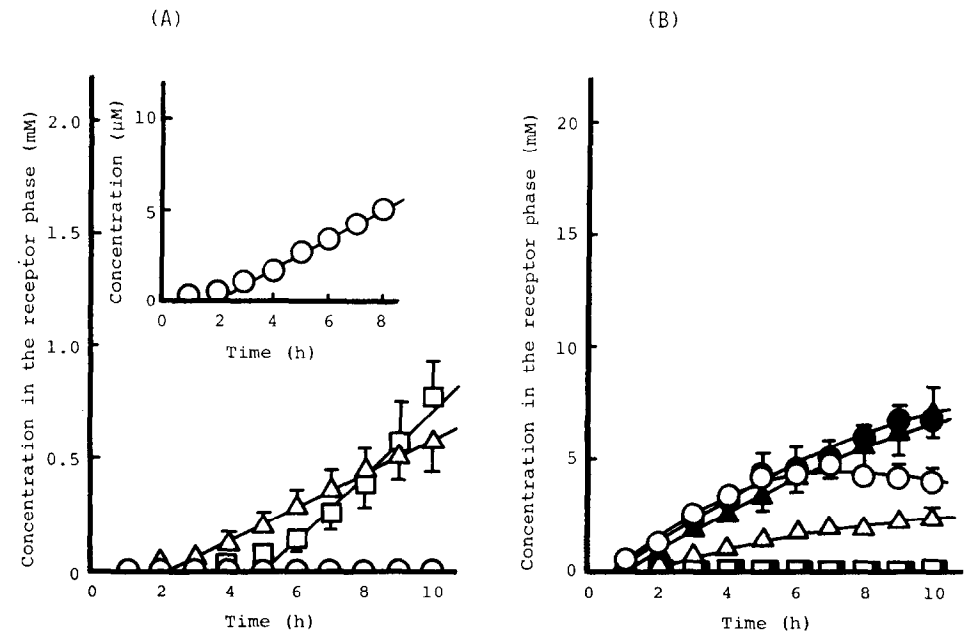


Fig. 4. Percutaneous penetration of Phenol red and 1-alkyl-4-methoxycarbonyl-2-pyrrolidones through a rat skin after their coapplication. A: Phenol red transfer. ○, **VII**; △, **VIII**; □, **IX**. Insert: an enlargement of the result of **VII**. B: enhancer transfer. ○, **VII**, ●, **IV** after application of **VII**; △, **VIII**; ▲, **V** after application of **VIII**; □, **IX**; ■, **VI** after application of **IX**. Vertical bars indicate S.E.M. and each point is the mean of at least 6 experiments.

to the receptor phase rapidly and **III** also showed high permeation (Fig. 2B). However, compound **III** was hardly detected in the receptor phase.

Effect of 1-alkyl-4-carboxy-2-pyrrolidones on in vitro transfer of Phenol red

A transfer of Phenol red was also observed after application with any 1-alkyl-4-carboxy-2-pyrrolidones (**IV**, **V** and **VI**) as shown in Fig. 3A. The promoting action of **V** and **VI** on Phenol red penetration was higher than that of **IV**. The constant appearance of Phenol red was observed after a lag time for 1.8, 3.3 and 5.5 h in **IV**, **V** and **VI**, respectively. However, these effects were lower and the lag times were longer than those of the corresponding 1-alkyl-2-pyrrolidones(**I**, **II** and **III**). Compounds **IV** and **V** were transferred slightly and **VI** showed little transfer (Fig. 3B).

Effect of 1-alkyl-4-methoxycarbonyl-2-pyrrolidones on in vitro transfer of Phenol red

Application of 1-alkyl-4-methoxycarbonyl-2-pyrrolidones(**VII**, **VIII** and **IX**) promoted the transfer of Phenol red as shown in Fig. 4A. Phenol red applied with **VIII** and **IX** showed high penetration with lag times of 2.1 and 4.9 h. Compound **VII** promoted dye penetration slightly with a lag time of 2.0 h. Compounds **VII** and **VIII** were transferred and most of them were hydrolyzed to 4-carboxy derivatives (**IV** and **V**) during the skin penetration. Compound **IX** was hardly transferred through the rat skin.

Effect of pyrrolidone derivatives on in vitro skin accumulation of Phenol red

In the same in vitro experiment, the skin accumulations of Phenol red and pyrrolidone derivatives at 10 h after coapplication are shown in Table 2. The amount in the skin tissue of Phenol red applied with pyrrolidone derivatives increased in comparison to that applied alone. Especially 1-hexyl- and 1-lauryl-substituted derivatives enhanced the accumulation of the dye highly. On the other hand, the enhancer showed high skin accumulation except for **I** and **VII**. The conversion of compounds **VII**, **VIII** and **IX** in the skin to **IV**, **V** and **VI** were also observed.

Effect of 1-alkyl-2-pyrrolidones on in vivo transfer of Phenol red

The plasma concentration and urinary accumulation of Phenol red, applied to a rat abdominal skin with or without 1-alkyl-2-pyrrolidones (**I**, **II** and **III**), are shown in Fig. 5. After application alone, Phenol red was not detected in both plasma and urine. However, Phenol red applied with II or III showed high plasma level and urinary excre-

TABLE 2

In vitro skin accumulation of Phenol red and 2-pyrrolidone derivatives at 10 h after coapplication

Compound	Skin accumulation at 10 h (μmol) [a]		
	Phenol red	Enhancer	Regenerating enhancer [b]
None [c]	0.20 ± 0.08 (3)	–	–
I	0.58 ± 0.05 (9)	9.46 ± 1.26 (8)	–
II	20.61 ± 0.85 (6)	126.36 ± 16.74 (9)	–
III	27.83 ± 19.73 (7)	64.58 ± 20.88 (6)	–
IV	0.65 ± 0.08 (6)	35.76 ± 6.51 (6)	–
V	2.49 ± 0.38 (11)	29.73 ± 7.39 (6)	–
VI	8.69 ± 1.69 (9)	28.48 ± 3.65 (6)	–
VII	0.48 ± 0.05 (9)	8.87 ± 2.42 (6)	54.66 ± 9.01 (6)
VIII	12.55 ± 3.38 (6)	31.88 ± 4.65 (6)	50.03 ± 14.93 (6)
IX	15.12 ± 2.83 (12)	22.39 ± 9.82 (9)	14.51 ± 3.71 (9)

[a] Means ± S.E.M. Numbers are given in parentheses.

[b] Skin accumulation of a regenerating 4-carboxy derivatives after application of 4-methoxycarbonyl derivatives (see text).

[c] Skin accumulation of phenol red at 24 h after its application alone.

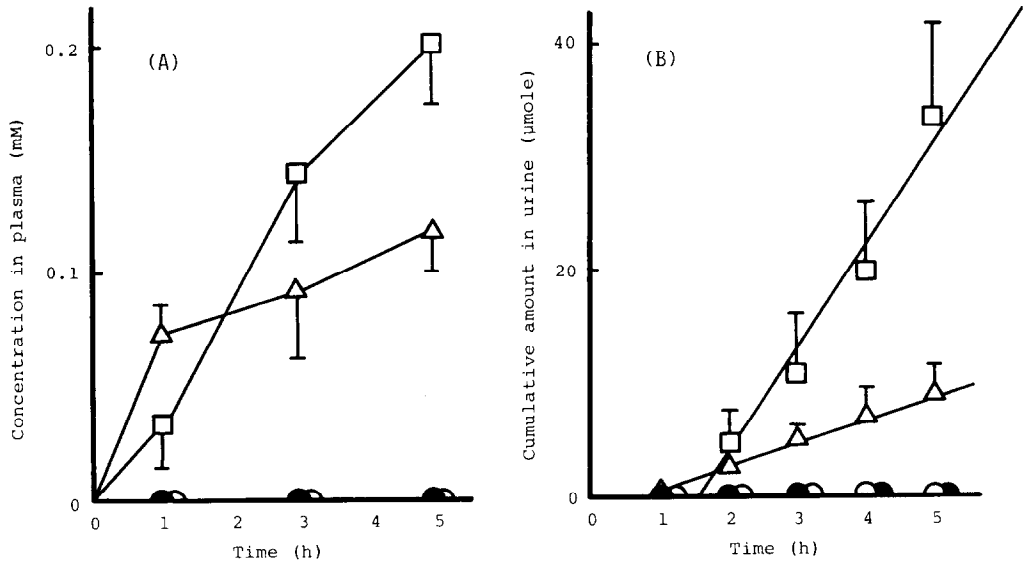


Fig. 5. Plasma concentration (A) and urinary accumulation (B) of Phenol red administered with or without 1-alkyl-2-pyrrolidones in a rat. ●, alone; ○, **I**; △, **II**; □, **III**. Vertical bars indicate S.E.M. and each point is the mean of at least 3 experiments.

tion. After application with **I**, there was little Phenol red in plasma and urine.

Discussion

The skin composite structure is indicated by the 3 distinct layers; the stratum corneum, the viable epidermis and the papillary layer of the dermis (Scheuplein and Blank, 1971). Among them, the lipophilic and ultradense characteristics of the stratum corneum are considered contribute to the low percutaneous absorption of particularly hydrophilic drugs (Flynn, 1979). In fact, Phenol red, as a model compound of a hydrophilic drug, transferred through a stripping skin which lost the stratum corneum layers partly although it could not penetrate through normal rat skin for 10 h (Fig. 1).

Some substances that could temporarily diminish the barrier of the skin have been reported, such as acetone, propylene glycol, dimethylsulfoxide, *N*,*N*-diethyl-*m*-toluamide and surface active agents (Poulsen, 1973; Idson, 1975; Windheuser, 1982). Compound I can be used with a variety of compounds including griseofulvin, theophylline, tetracycline, ibuprofen and betamethasone 17-benzoate to promote penetration and to establish a reservoir of drug in the stratum corneum (Barry, 1983; Akhter and Barry, 1985; Bennet et al., 1985). Recently, 1-dodecylazacycloheptan-2-one was developed as a potential penetration enhancer without severe side effect (Stoughton and McClure, 1983). Two enhancers have the similar ring structure but different alkyl chains.

In the present study, we prepared 8 derivatives of **I** and investigated their promoting action on percutaneous absorption of Phenol red in relation to their lipophilicity and absorption behavior. Phenol red suspension in isopropyl myristate containing enhancer was used as a formulation to investigate the maximum penetration of dye through a modified skin by enhancer.

Any pyrrolidone derivative enhanced a penetration of Phenol red (Figs. 2–4). A dipolar aprotic nature such as that of pyrrolidones is suggested to be of importance in their mode of action(Hadgraft, 1984).

When the promoting effect of three 1-alkyl-2-pyrrolidones (**I**, **II** and **III**) was compared, lipo-

philic compounds, **II** and **III**, showed superior enhancing effect on Phenol red penetration to **I** (Fig. 2). Their hydrophobicity is considered to be necessary to maintain an effective concentration of enhancer in the stratum corneum or its hydrophobic contents. In fact, **II** and **III** showed higher skin accumulation than **I** (Table 2).

Compound **I** transferred rapidly. **II** showed gradual penetration and little transfer of **III** was observed (Fig. 2B). Thus, introduction of a lipophilic group into pyrrolidone is expected to make it a potential enhancer without systemic toxicity because of its non-penetrating characteristics.

A carboxy group or its methylester was introduced as a hydrophilic function to each 1-alkyl-2-pyrrolidone. Also in this series, their physicochemical properties and degree of enhancing effect largely depend on the introduced alkyl group. The promoting activity was found in the case of simple fatty acids (Cooper, 1984). However, the introduction of hydrophilic function decreased the promoting effect and prolonged the lag time (Fig. 3). Carboxy derivatives showed higher melting point, suggesting that they are thermodynamically unavailable (Table 1). These disadvantageous characteristics were considered to decrease penetration of enhancer to the stratum corneum and reduce the promoting effect. Methoxycarbonyl derivatives showed higher effect than carboxy derivatives but had slightly disadvantageous characteristics in comparison with corresponding 1-alkyl-2-pyrrolidones (Fig. 4). It is worth noticing the appearance of carbonyl derivatives from **VII** and **VIII** by hydrolysis during penetration.

The present enhancers also increased the skin accumulation of Phenol red (Table 2). The enhancement of a dye transfer resulted in an increase of its accumulation. However, an accumulation of enhancer showed no relation with its absorption behavior. Lipophilic compounds (**III, VI** and **IX**) were strongly accumulated in the skin. The hydrolysis of **VII, VIII** and **IX** in the skin was confirmed.

The enhancing effect of 1-alkyl-2-pyrrolidone derivatives (**I, II** and **III**) was supported by an in vivo absorption experiment. Phenol red administered with compounds **II** and **III** showed high appearance in the plasma and urine although it was not detected when administered alone or with **I** (Fig. 5).

Thus, from these results, we conclude that the physicochemical properties and the transdermal absorption behavior of the enhancer itself are among the important factors that affect the pattern of promoting actions and side effects.

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