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Enhancing effect of pyrrolidone derivatives on transdermal drug delivery II. Effect of application concentration and pre-treatment of enhancer

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Summary

We compared the enhancing effects of 1-methyl- (**I**), 1-hexyl- (**II**) and 1-lauryl-2-pyrrolidone (**III**) on the penetration of phenolsulfonphthalein (Phenol red) as a model for a non-absorbable drug. Using the in vitro penetration technique and excised rat skin, the enhancers were applied at various concentrations. An increase in enhancer concentration was found to increase the flux and skin accumulation, and shorten the lag time for steady-state penetration of Phenol red. The enhancing effects of **II** and **III** ceased at 0.1 mmol/ml. Penetration for enhancers was found to increase with their own concentrations. Pre-treatment with enhancer for 5 h shortened the lag time for steady-state penetration of Phenol red. Removal of **II** from the donor side after pre-treatment decreased its enhancing effect. Enhancer **III** still showed an effect after removal.

Introduction

Recently, percutaneous drug delivery has attracted a great deal of interest not only in local chemotherapy but also in systemic chemotherapy using controlled-release technology (Shaw, 1982; Chien, 1983). However, most drugs will not penetrate through the skin at rates high enough for therapeutic efficacy because of the lipophilic and ultradense characteristics of the stratum corneum (Flynn, 1979; Higuchi et al., 1985). Numerous attempts have been reported to improve permeation by use of pharmaceutical excipients. A promising approach is the use of transdermal penetration enhancers. Some substances that could diminish the barrier properties of the skin have been reported, such as acetone, propylene glycol, dimethyl sulfoxide, *N,N*-diethyl-*m*-toluamide and surface-active agents (Poulsen, 1973; Idson, 1975; Windheuser, 1982; Hadgraft, 1984).

1-Methyl-2-pyrrolidone (**I**) may be used with a variety of compounds including griseofulvin, theophylline, tetracycline, ibuprofen and betametha-

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sone 17-benzoate to promote penetration and to establish a reservoir of drug in the stratum corneum (Barry, 1983; Akhter and Barry, 1985; Bennett et al., 1985). Recently, 1-dodecylazacycloheptan-2-one (Azone®) was developed by Nelson Research (California, U.S.A.) as a potential penetration enhancer without severe side effects (Stoughton and McClure, 1983). The two enhancers have a similar ring structure but different alkyl chains. This difference may influence their enhancing characteristics.

In our preceding study (Sasaki et al., 1988), nine pyrrolidone derivatives were prepared and investigated for their enhancing effects on percutaneous absorption of phenolsulfonphthalein (Phenol red) as a model of non-absorbable compounds. In the present study, the effects of varying concentrations of 1-methyl- (**I**), 1-hexyl- (**II**) and 1-lauryl-2-pyrrolidone (**III**) on self-penetration and penetration of Phenol red were examined by using an in vitro technique with rat skin as a model membrane. The effect of pre-treatment with **II** and **III** on Phenol red penetration was also investigated.

Materials and Methods

Chemicals

Compound **I** and Phenol red were obtained from Nacalai Tesque (Kyoto, Japan). Compounds **II** and **III** were prepared by a routine method (Zienty and Steahly, 1947). All other reagents were of reagent grade.

In vitro penetration 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. Hair was removed from rats with an animal clipper and a shaver 24 h before the experiment. Animals were killed with pentobarbital, given intraperitoneally. The skin was excised and mounted in the diffusion cell. 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) in the presence or absence of pyrrolidone derivatives (2, 0.5 and 0.1 mmol/ml). The pyrrolidone derivatives were dissolved in isopropyl myristate. These test formulations were gently applied on the donor side of the skin surface which had an available diffusion area of 6.8 cm^2. The diffusion cell was placed in a thermostated chamber maintained at 32°C and the receptor phase was agitated using a magnetic stirrer. At appropriate intervals, samples of the receptor fluid were withdrawn for 10 h.

In the pre-treatment experiment, compounds **II** and **III** (2 and 0.5 mmol/ml) in isopropyl myristate were applied on the skin mounted on the diffusion cell for 5 h. The formulation on the donor side was mixed with 200 mg Phenol red and re-applied on the skin. The penetration profile of Phenol red was monitored.

In the removal experiment, after pre-treatment with enhancer (2 and 0.5 mmol/ml) for 16 h, receptor fluid and the formulation on the donor side were removed. Fresh portions of buffered saline and 1 ml Phenol red suspension in isopropyl myristate without enhancers were added on the receptor and donor sides, respectively. The penetration profiles of Phenol red and enhancer were monitored.

At the end of the transfer period, the donor phase was washed with water and skin was removed from the diffusion cell, followed by homogenization in 50 ml of water using a Polytron Homogenizer® (Ikemotorika Kogyo, Tokyo, Japan). The homogenate was diluted with an equal volume of methanol, shaken and filtered on paper filters (Toyo Roshi, Tokyo). The filtrate was used for HPLC assay.

Analysis

The pyrrolidone derivatives were assayed using an HPLC system (LC-5A pump, SIL-1A injector, Shimadzu, Kyoto) equipped with a variable-wavelength ultraviolet absorbance detector (SPD-2A, Shimadzu) in reverse-phase mode. The stationary phase used was a Cosmosil $5C_{18}$ packed column (diameter 4.6 mm, length 150 mm, Nacalai Tesque), the peak being detected at 205 nm. The column was run at room temperature. Mixtures of methanol-water (**I**, 5 : 95; **II**, 55 : 45; **III**, 85 : 15,

v/v) were used as the mobile phase at a flow rate of 1.0 ml/min. The mobile phase was filtered by passage through a 0.45 μm pore size membrane filter (Toyo Roshi). Standard solutions were chromatographed and calibration curves were constructed on the basis of peak area measurements.

Phenol red was assayed using a spectrophotometer (UV 110, Hitachi, Tokyo) at 550 nm under alkaline conditions by dilution with 1 M NaOH.

Mathematical analysis

The appearance of Phenol red in a receptor phase after pre-treatment of II and III was analysed using Fick's equation as modified slightly in accordance with Okamoto et al. (1986). The total amount of drug Q which appeared in the receptor phase through the skin in time t can be represented as follows;

$$Q = A(lC_s)\left[(D/l^2)t - (1/6) - (2/\pi^2) \times \sum_{n=1}^{\infty} \{(-1)^n(1/n^2)\exp(-n^2\pi^2(D/l^2)t)\}\right] \quad (1)$$

where A, l, C_s and D denote the area for application, thickness of membrane, skin surface concentration of penetrant and diffusion constant, respectively. Since there is some difficulty in the accurate determination of the thickness of the real diffusion barrier, two parameters involving the skin thickness, namely, the diffusion parameter D/l^2 and skin surface concentration parameter of penetrant, lC_s, were determined. The permeability rate, dQ/dt $((D/l^2)(l/C_s))$, and lag time $(1/(6D/l^2))$ for penetrant transfer through skin were calculated.

The above parameters were calculated on a microcomputer using a non-linear least-squares method (Yamaoka et al., 1981).

Results and Discussion

The penetration profiles of Phenol red through rat skin after co-application with 1-alkyl-2-pyr-

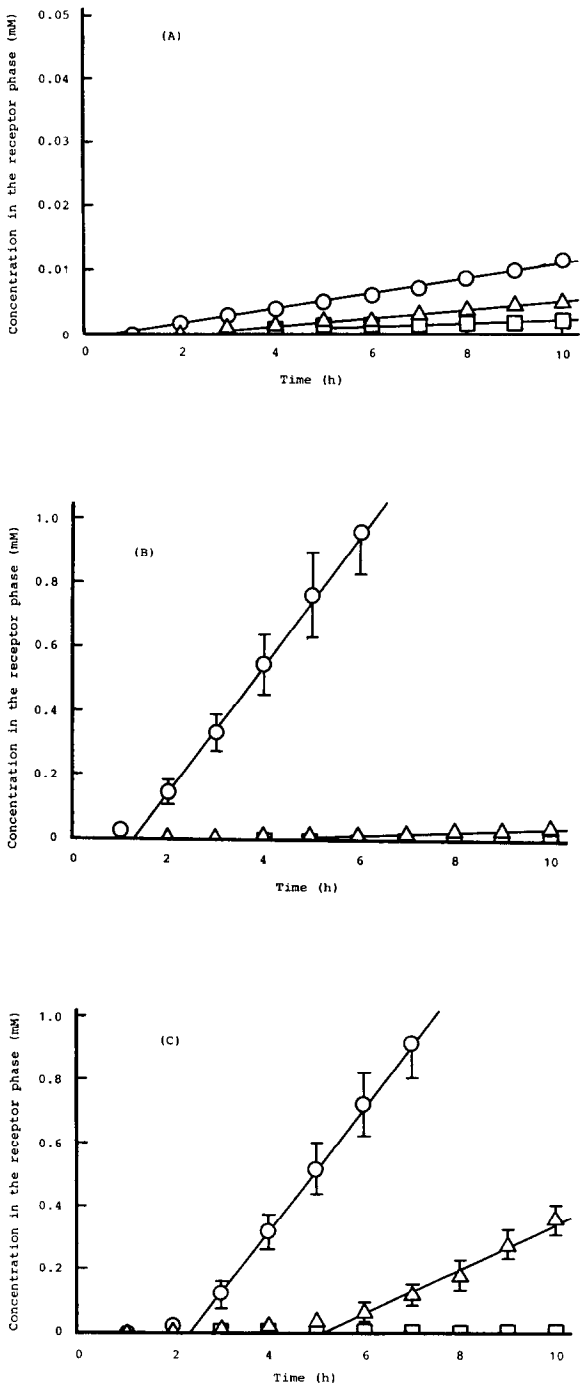

Fig. 1. Percutaneous penetration of Phenol red after co-application with pyrrolidone derivatives, **I** (A), **II** (B) and **III** (C), at various concentrations. (○) 2, (△) 0.5 and (□) 0.1 mmol/ml. Vertical bars indicate standard errors and each point is the mean of at least three experiments.

TABLE 1

In vitro skin accumulation of Phenol red and pyrrolidone derivatives at 10 h after co-application

Enhancer	Concentration (mmol/ml)	Skin accumulation at 10 h (μmol) [a]	
		Phenol red	Enhancer
None		0.20 ± 0.08 (3) [b]	-
I	2	0.58 ± 0.05 (9)	9.46 ± 1.26 (8)
	0.5	0.43 ± 0.05 (3)	3.89 ± 1.05 (3)
	0.1	0.30 ± 0.06 (4)	0.93 ± 0.24 (4)
II	2	20.61 ± 0.85 (6)	126.36 ± 16.74 (9)
	0.5	1.43 ± 0.36 (4)	11.87 ± 1.88 (4)
	0.1	0.25 ± 0.12 (3)	1.48 ± 0.38 (3)
III	2	27.83 ± 19.73 (7)	64.58 ± 20.88 (6)
	0.5	5.34 ± 0.77 (7)	12.11 ± 1.38 (6)
	0.1	0.46 ± 0.02 (3)	0.55 ± 0.33 (3)

[a] Means ± S.E. Number of trials are given in parentheses.
[b] Skin accumulation of Phenol red 24 h after its application alone.

rolidones (**I–III**) at various concentrations (2, 0.5 and 0.1 mmol/ml) are shown in Fig. 1 (A–C). Skin accumulation of Phenol red and enhancer at 10 h is summarized in Table 1. Phenol red suspension in isopropyl myristate was used as a formulation to determine maximum penetration of the dye. Isopropyl myristate was used as a model for lipophilic vehicles. Phenol red applied alone did not penetrate into the receptor phase. On the other hand, pyrrolidone derivatives effectively enhanced both penetration and skin accumulation of Phenol red. In particular, lipophilic compounds such as **II** and **III** showed a strong enhancing effect. This was found to be dependent on the concentration of enhancer applied in the formulation. The enhancing effect ceased at 0.1 mmol/ml. It has been reported that the concentration of absorption enhancer in a formulation markedly influences the promotion of transdermal drug delivery (Barry, 1983; Stoughton and McClure, 1983; Chow et al., 1984). All penetration profiles for Phenol red through rat skin consisted of a lag phase followed by a linear rise. The lag time was prolonged by decreasing concentration of enhancers.

Fig. 2 (A and B) depicts the appearance of **I** and **II** in penetration experiments. Enhancer penetration through the skin was also concentration-dependent. Compound **III** showed little penetration due to its high lipophilicity and poor aqueous solubility (data not shown). Skin accumulation of all enhancers, **I–III**, showed a concentration dependence (Table 1). These results indicated that the amount of enhancer present in the skin was an important factor in the enhancing effect. An increase in quantity of each enhancer in the skin increased the extent of enhancement.

Fig. 3 (A and B) and Table 2 illustrate penetration and skin accumulation of Phenol red, respectively, after pre-treatment of excised rat skin with

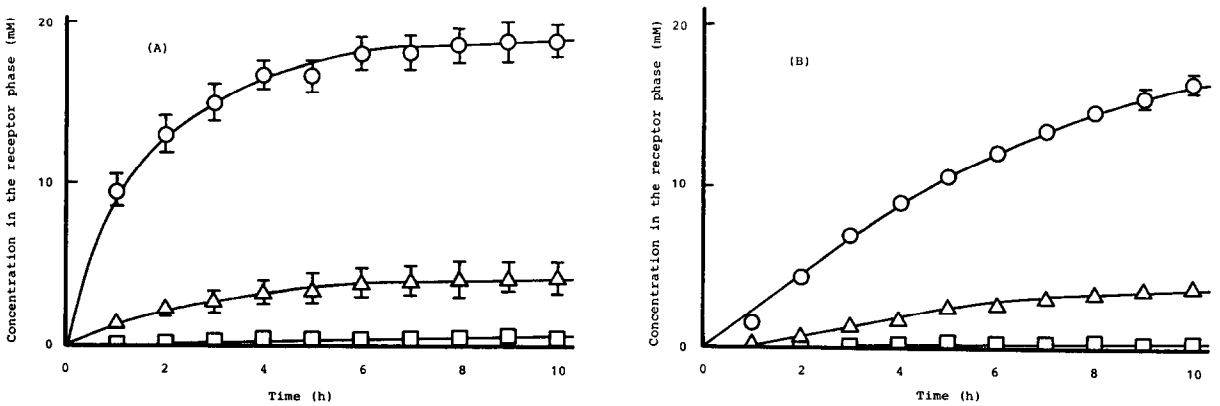

Fig. 2. Percutaneous penetration of pyrrolidone derivatives, **I** (A) and **II** (B), after application at various concentrations. (○) 2, (△) 0.5 and (□) 0.1 mmol/ml. Vertical bars indicate standard errors and each point is the mean of at least three experiments.

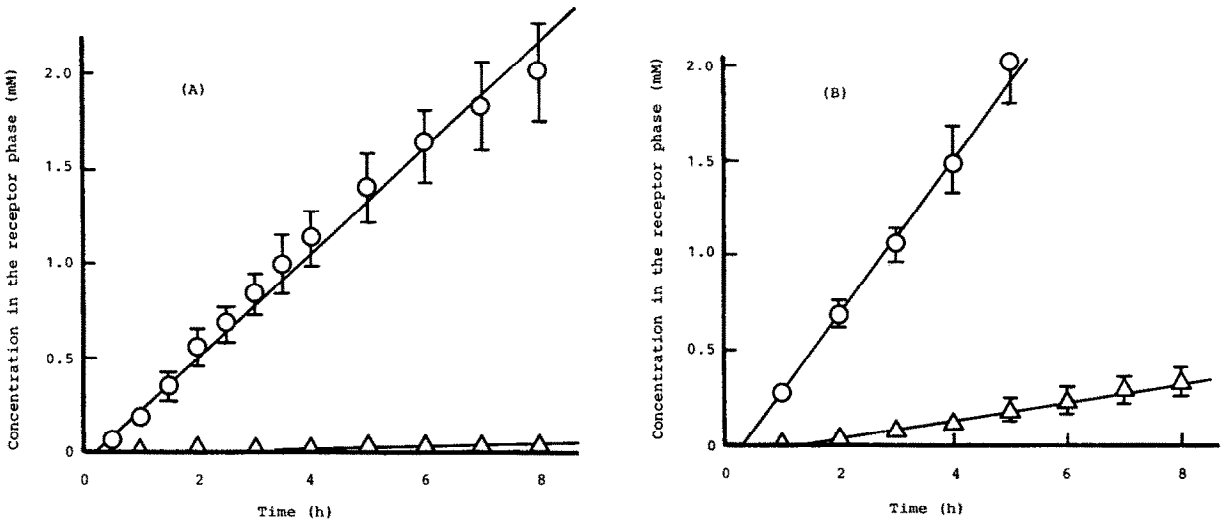

Fig. 3. Percutaneous penetration of Phenol red after pre-treatment with pyrrolidone derivatives, **II** (A) and **III** (B), at various concentrations for 5 h. (○) 2 and (△) 0.5 mmol/ml. Vertical bars indicate standard errors and each point is the mean of at least three experiments.

II and **III** for 5 h. The enhancing effect of pyrrolidone derivatives after pre-treatment was still dependent on their concentration. The above profiles for Phenol red penetration were analysed using Fick's equation and the parameters evaluated are listed in Table 3. Enhancer concentration influenced not only the skin surface concentration parameter (lC_s) but also that of diffusion (D/l^2). The pre-treatment with enhancer reduced the lag time for steady-state penetration of Phenol red.

Chow et al. (1984) and Morimoto et al. (1986) demonstrated Azone® to have a long lag time in enhancing the dermal penetration of triamcinolone acetonide and 5-fluorouracil, which was not found after pre-treatment with enhancer. The apparent lag times for Phenol red penetration after pre-treatment and coapplication of enhancer, summarized in Table 4, were determined graphically. The lag time obtained graphically was comparable to that calculated from the diffusion equation (Table 3). The difference between the apparent lag times for pre-treatment and co-application was calculated as ELT. The apparent lag time after pre-treatment is considered to be related to the process of diffusion of Phenol red through the

TABLE 2

In vitro skin accumulation of Phenol red and pyrrolidone derivatives at 8 h after pre-treatment of pyrrolidone derivatives

Enhancer	Concentration (mmol/ml)	Skin accumulation at 8 h (μmol) [a]	
		Phenol red	Enhancer
II	2	14.16 ± 1.99 (5)	73.86 ± 13.16 (5)
	0.5	1.08 ± 0.24 (6)	14.87 ± 2.05 (5)
III	2	14.86 ± 2.80 (5)	31.22 ± 5.95 (4)
	0.5	3.13 ± 0.54 (8)	13.07 ± 2.30 (7)

[a] Means ± S.E. Number of trials are given in parentheses.

TABLE 3

Parameters for percutaneous penetration of Phenol red after pre-treatment of pyrrolidone derivatives

Enhancer	Concentration (mmol/ml)	Parameters [a]			
		lC_s (μmol/cm²)	D/l^2 (1/h)	dQ/dt (μmol/cm² per h)	LT (h)
II	2	2.94	0.73	2.13	0.13
	0.5	0.43	0.10	0.04	1.66
III	2	6.42	0.47	3.03	0.35
	0.5	3.37	0.11	0.38	1.49

[a] Values were determined using a modified version of Fick's equation, Eqn 1. lC_s, skin surface concentration parameter of penetration; D/l^2, diffusion parameter; dQ/dt, transfer rate; LT, lag time. See text.

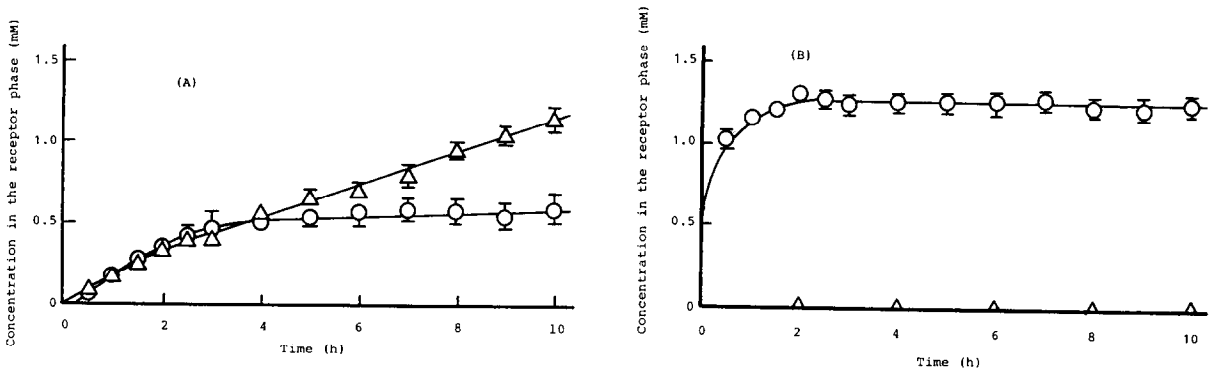

Fig. 4. Percutaneous penetration of Phenol red (A) and appearance of enhancer (B) in receptor phase after pre-treatment for 16 h and removal of pyrrolidone derivatives. (○) **II**, (△) **III**. Concentration of enhancer: 2 mmol/ml. Vertical bars indicate standard errors and each point is the mean of at least three experiments.

skin modified by enhancer. Reduction in the lag time is a result of the diffusion rate being promoted. On the other hand, the meaning of ELT is complicated, however, it reflects mainly the time during which enhancer penetrates into the skin and shows a constant enhancing effect. A decrease in concentration of enhancer resulted in the prolongation of not only the lag time but also ELT. It is worth noting that compound **II** had a shorter ELT than **III**, since reduction of the ELT appears to be important for the development of a useful transdermal drug delivery system. The long lag time for application of **III** may be due to its slow penetration into the skin.

The effect of removing enhancer after pre-treatment is shown in Fig. 4 (A and B). In this experiment, the pre-treatment period was 16 h so that skin was completely modified by enhancer. Removal of **II** resulted in a decrease in the enhancing effect. Rapid leakage of **II** from the skin into the receptor phase was observed. This suggested that the enhancing effect of **II** was reversible. The co-existence of Phenol red and a sufficient amount of enhancer in the skin (probably the stratum corneum) might be responsible for the observed enhancement of penetration. An enhancing effect still remained after the removal of **III**. The appearance of **III** was not detected in the receptor phase. Compound **III** remaining in the skin might show an enhancing effect as a result of the longer period of time needed to remove **III** from skin in comparison with **II**.

Thus, from the present results, we conclude that the physicochemical properties and behavior of the enhancer itself in the skin are among the most important factors that affect the pattern of promotion.

TABLE 4

Apparent lag times for Phenol red appearance after co-application and pre-treatment of pyrrolidone derivatives

Enhancer	Concentration (mmol/ml)	Apparent lag time (h) [a]		ELT [b] (h)
		Co-application	Pre-treatment	
II	2	1.05 ± 0.13 (9)	0.23 ± 0.04 (5)	0.82
	0.5	2.68 ± 0.17 (4)	1.21 ± 0.14 (4)	1.47
III	2	2.14 ± 0.09 (9)	0.38 ± 0.07 (7)	1.76
	0.5	4.70 ± 0.38 (6)	1.24 ± 0.34 (6)	3.46

[a] Means ± S.E. Number of trials are given in parentheses.
[b] Values were determined by subtraction of apparent lag time in pre-treatment experiment from that in co-application.

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