Enhancing Effect of Pyrrolidone Derivatives on Transdermal Penetration of Phenolsulfonphthalein and Indomethacin from Aqueous Vehicle

Hitoshi SASAKI,* Masaki KOJIMA, Junzo NAKAMURA and Juichiro SHIBASAKI

Faculty of Pharmaceutical Sciences, Nagasaki University, Bunkyo-machi 1–14, Nagasaki 852, Japan. *Received July 21, 1989*

We investigated the enhancing effect of three alkyl-2-pyrrolidones on transdermal penetration of phenolsulfonphthalein (phenol red) and indomethacin from an aqueous vehicle by using an *in vitro* technique with excised rat skin. The enhancers included 1-methyl- (I), 1-hexyl- (II) and 1-lauryl-2-pyrrolidone (III). These derivatives effectively enhanced the penetration and skin accumulation of phenol red and indomethacin. Lipophilic enhancers such as II and III showed particularly high enhancing effects. The penetration profiles of phenol red and indomethacin showed a lag phase followed by a linear increase. Compounds II and III showed long lag times. The enhancer penetration was also determined. Compounds I and II showed a slight penetration. Compound III showed little penetration but high skin accumulation.

Keywords percutaneous absorption; pyrrolidone derivative; *in vitro* experiment; transdermal drug delivery; aqueous vehicle; penetration enhancer; phenolsulfonphthalein; phenol red; indomethacin; skin accumulation

Recently, percutaneous drug delivery has attracted much interest in local and systemic chemotherapy.[1] However, most drugs can not penetrate the skin at rates high enough for therapeutic efficacy. Numerous attempts have been made to deliver drugs across the skin by means of pharmaceutical approaches. One promising approach is the use of transdermal penetration enhancers, which should be applicable to most drugs.[2]

In the previous report, we demonstrated the enhancing effect of pyrrolidone derivatives on transdermal penetration of phenolsulfonphthalein (phenol red) from isopropyl myristate, as a model of a lipophilic vehicle.[3] It is well known that the vehicle is important for determining the penetration of a penetrant and the promoting effect of an enhancer.[4] In the present study, the transdermal penetration of phenol red and indomethacin from an aqueous vehicle was investigated after application with enhancers such as 1-methyl- (I), 1-hexyl- (II) and 1-lauryl-2-pyrrolidones (III).

Experimental

Materials Compound I and phenol red were obtained from Nacalai Tesque, Inc., Kyoto, Japan. Compounds II and III were prepared by a usual method.[5] All other reagents were of reagent grade.

Apparent Partition Coefficient and Solubility Apparent partition coefficients of indomethacin were determined in a chloroform (5 ml)–citrate buffer (pH 4.0, 10 mM, 35 ml) system at 0.5 mM after shaking for 1 h and standing for 1 d at 32 °C. A chloroform (5 ml)–water (35 ml) system was used for phenol red because of instability in citrate buffer. Before use, chloroform and the aqueous solutions were saturated with the relevant aqueous or organic phase.

The solubilities of phenol red and indomethacin were determined at 32 °C by suspending excess amounts of them in hexane or distilled water, followed by filtration (0.45 μm pore size membrane filter, Nihon Millipore Kogyo K.K., Yonezawa, Japan) and analysis.

***In Vitro* Penetration Experiment through Rat Skin** The *in vitro* diffusion cell was similar to the type used by Loftsson and Bodor.[6] The diffusion membranes were full-thickness abdominal skin of male Wistar albino rats weighing 250—300 g. The hair was removed with an animal clipper and a shaver at 24 h before the experiment. The animal was 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) and indomethacin (150 mg) in distilled water (1 ml) containing pyrrolidone derivatives (2 mmol). Compound I was dissolved in water. Compounds II and III were suspended in water by sonication. 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 stirred with a magnetic stirrer. At appropriate intervals for 10 h, samples of the receptor fluid were withdrawn.

At the end of a transfer period, the donor phase was washed with water and the skin was removed from the diffusion cell and homogenized in 50 ml of water with Polytron Homogenizer® (Ikemotorika Kogyo Co., Ltd., Tokyo, Japan). The homogenate was diluted with an equal volume of methanol, shaken and filtered through filter paper (Toyo Roshi Co., Ltd., Tokyo, Japan). The filtrate was used for high performance liquid chromatography (HPLC) assay.

Analysis The pyrrolidone derivatives and indomethacin were determined by the use of an HPLC system (LC-5A pump, SIL-1A injector, Shimadzu Co., Ltd., Kyoto, Japan) equipped with a variable-wavelength ultraviolet (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, Nacalai Tesque, Inc.) and the peak was detected at 205 nm for pyrrolidone derivatives and 265 nm for indomethacin. The column was used at room temperature. Mixtures of methanol–water (I, 5 : 95; II, 55 : 45; III, 85 : 15, v/v) were used as the mobile phase for pyrrolidone derivatives at flow rate of 1.0 ml/min. A mixture of methanol–10 mM citrate buffer (75 : 25, v/v) was used as the mobile phase for indomethacin. The mobile phases were filtered by passing them through a 0.45 μm pore size membrane filter (Toyo Roshi Co., Ltd.). The standard solutions were chromatographed and calibration curves were constructed on the basis of peak-area measurements.

The phenol red was assayed with a spectrophotometer (UV 110, Hitachi Co., Ltd., Tokyo, Japan) at 550 nm under alkaline conditions by diluting with 1 M NaOH.

Results and Discussion

The enhancing effect of pyrrolidone derivatives on penetration of phenol red and indomethacin in an aqueous vehicle was investigated by using an *in vitro* technique with excised rat skin. A hydrophilic dye, phenol red, was used as a model of a non-absorbable drug. Indomethacin, which was used as a lipophilic drug, has been used clinically as a non-steroidal antiinflammatory agent by means of not only systemic administration but also topical application. In fact, indomethacin (apparent partition coefficient; 500) showed higher lipophilicity than phenol red (apparent partition coefficient; 0.0001). Indomethacin was also soluble in hexane (0.0022 mM) and water (0.039 mM) although phenol red was insoluble in hexane and soluble in water (0.47 mM). The suspension of phenol red and indomethacin in distilled water was used as a formulation to determine the maximum penetration of penetrant through the skin.

Penetration profiles of penetrants and pyrrolidone de-

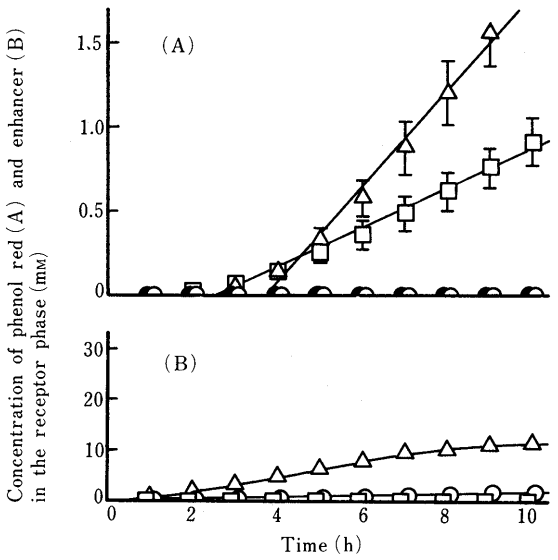

Fig. 1. Percutaneous Penetration of Phenol Red (A) and Pyrrolidone Derivatives (B) through Rat Skin after Their Application in an Aqueous Vehicle

●, none; ○, I; △, II; □, III. Vertical bars indicate standard errors and each point is the mean of at least three experiments.

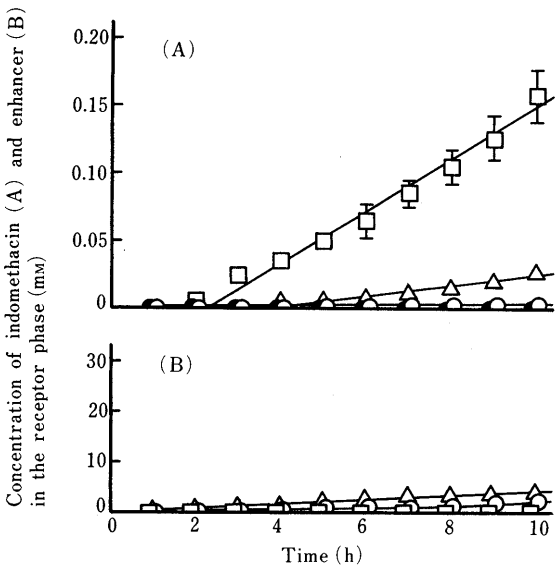

Fig. 2. Percutaneous Penetration of Indomethacin (A) and Pyrrolidone Derivatives (B) through Rat Skin after Their Application in an Aqueous Vehicle

●, none; ○, I; △, II; □, III. Vertical bars indicate standard errors and each point is the mean of at least three experiments.

rivatives after their co-application in an aqueous vehicle are shown in Fig. 1 for phenol red and Fig. 2 for indomethacin. The penetration profiles of penetrants showed a lag phase followed by a linear increase. The lag time and transfer rate were calculated graphically and are summarized in Table I. Indomethacin showed penetration but phenol red showed no penetration after application alone. Lipophilic pyrrolidone derivatives, II and III, enhanced the penetrations of both hydrophilic dye and lipophilic drug. Compound I showed little enhancing effect in an aqueous vehicle. However, a higher enhancing effect of I in isopropyl myristate was observed with high penetration of I itself in a previous report.[3] We also demonstrated that a reduction of

TABLE I. Transdermal Absorption Characteristics of Phenol Red and Indomethacin Applied with Pyrrolidone Derivatives in an Aqueous Vehicle

Compd.	Phenol red		Indomethacin	
	Lag time (h)	Transfer rate (nmol/h)	Lag time (h)	Transfer rate (nmol/h)
None	nd (4)	nd (4)	1.09±0.42 (5)	6± 1 (5)
I	1.42±0.44 (3)	17± 14 (3)	1.08±0.44 (3)	16± 2 (3)
II	3.89±0.12 (7)	14163±1913 (7)	3.93±0.21 (6)	205± 35 (6)
III	2.88±0.17 (3)	5996± 839 (3)	2.25±0.25 (5)	910±104 (5)

Means±standard error of the mean. Numbers of trials are given in parentheses. Lag time and transfer rate were determined graphically. nd: not detected in the receptor phase for 10 h.

TABLE II. *In Vitro* Skin Accumulation of Phenol Red, Indomethacin and Pyrrolidone Derivatives at 10 h after Application in an Aqueous Vehicle

Compd.	Phenol red		Indomethacin	
	Penetrant (μmol)	Enhancer (μmol)	Penetrant (μmol)	Enhancer (μmol)
None	nd (4)	—	0.14±0.05 (5)	—
I	0.48±0.13 (3)	16.06±1.74 (3)	1.41±0.54 (3)	7.63±0.36 (3)
II	16.60±4.42 (7)	36.39±5.96 (7)	14.95±0.79 (6)	30.37±2.38 (6)
III	10.81±0.43 (3)	38.88±5.18 (3)	4.36±0.35 (5)	12.93±3.00 (3)

Means±standard error of the mean. Numbers of trials are given in parentheses. nd: not detected in the skin at 10 h.

enhancer penetration decreased the enhancing effect. Therefore, the inactivity of I can be explained by poor penetration of I from the aqueous vehicle into the skin. Thus, the vehicle may influence not only penetrant penetration but also enhancer penetration. Lipophilic compounds II and III had a long lag time for steady-state penetration of penetrants.

The skin accumulation of phenol red, indomethacin and pyrrolidone derivatives at 10 h after their application is summarized in Table II. Pyrrolidone derivatives enhanced not only penetration but also skin accumulation of the hydrophilic dye and the lipophilic drug, suggesting the usefulness of the aqueous vehicle for topical therapy. Compounds II and III showed particularly high enhancing effects.

Although additional work is necessary, it is suggested that adequate selection of drug, vehicle and enhancer is important for the development of useful transdermal drug delivery systems.

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