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Enhancing effect of combining two pyrrolidone vehicles on transdermal drug delivery

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Abstract—The enhancing effect of combining 1-methyl-2-pyrrolidone (MP) and 1-lauryl-2-pyrrolidone (LP) as the vehicles for transdermal penetration of phenolsulphonphthalein (phenol red) has been investigated by using an in-vitro technique with excised rat skin. LP had a higher enhancing effect on the penetration of phenol red than MP, but there was a long lag time before steady-state penetration was attained. A potent effect with a shorter lag time was obtained when MP and LP were used together. This potentiation was maintained when the concentration of MP was decreased by 95%. The combined vehicle also enhanced the skin accumulation of phenol red. MP promoted the rapid penetration of LP into the skin and potentiated the enhancing effect of LP on the penetration of phenol red and thereby shortened the lag time. The combined vehicle also enhanced the penetration of the hydrophilic anticancer agent, 5-fluorouracil.

Several organic solvents such as dimethyl sulfoxide, *N*, *N*-dimethylformamide, *N*, *N*-dimethylacetamide, 1-methyl-2-pyrrolidone, propylene glycol and ethanol are known to enhance the transdermal penetration of drugs (Idson 1975; Hadgraft 1984; Bennett et al 1985). Several amphiphilic molecules such as alkyl sulfoxide, phosphine oxide, sugar esters and surfactants also have an enhancing effect (Barry 1983; Chien 1983; Hadgraft 1984). New amphiphilic molecules such as 1-dodecylazacycloheptan-2-one (Azone, Stoughton 1982; Stoughton & McClure 1983), *N*,*N*-diethyl-*m*-toluamide (Windheuser et al 1982), amides of cyclic amines (Mirejovsky & Takruri 1986), pyrrolidone derivatives (Sasaki et al 1988), unsaturated cyclic ureas (Wong et al 1988), alkyl *N*,*N*-dialkyl-substituted amino acetates (Wong et al 1989) and 1-alkyl- or 1-alkenylazacycloalkanone derivatives (Okamoto et al 1988) have been developed because of their potency in enhancing the penetration of a wide variety of drugs. Recently, it has been demonstrated that a binary vehicle combining organic solvent and amphiphilic compound has a high enhancing effect (Wotton et al 1985; Hoelgaard & Møllgaard 1985). In the present study, the enhancing effect of a combined vehicle of 1-methyl-2-pyrrolidone (MP) as organic solvent and 1-lauryl-2-pyrrolidone (LP) as the amphiphilic compound on penetration of phenolsulphonphthalein (phenol red) has been investigated by using an in-vitro technique and excised rat skin.

Materials and methods

Materials. Phenol red and 1-methyl-2-pyrrolidone (MP) were obtained from Nacalai Tesque, Inc., Kyoto, Japan. 1-Lauryl-2-pyrrolidone (LP) was prepared according to Zienty & Steahly (1947). All other reagents were of reagent grade.

In-vitro penetration through rat skin. The in-vitro diffusion cell was similar to that used by Loftsson & Bodor (1981). The membranes were full-thickness abdominal skins of male Wistar albino rats, 250–300 g, which had been depilated by shaver 24 h before the experiment. After death following pentobarbitone, (overdose i.p.), animals had the skin 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 sulphate (100 ppm). Test formulations were prepared by suspending phenol red (200 mg) or 5-fluorouracil (150 mg) in isopropyl myristate (1 mL) containing MP (0, 0·1, 0·5, 1 or 2 mmol mL^{-1}) and LP (0 or 2 mmol mL^{-1}). 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 stirred by a magnetic stirrer. At appropriate intervals, samples of the receptor fluid were withdrawn over 10 h. Concentrations of pyrrolidone derivatives and phenol red in the samples were determined by HPLC and spectrophotometry, respectively. The sample for 5-fluorouracil was acidified with 0·1 M HCl and washed with CH_2Cl_2. The aqueous layer was used for HPLC assay.

At the end of a transfer period, the donor phase was washed with water. 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 being used for HPLC assay.

Analysis. 5-Fluorouracil, MP and LP were determined by an 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 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 the enhancer and at 265 nm for the 5-fluorouracil. The column was used at room temperature (20°C). Mixtures of methanol–water (MP, 5:95; LP, 85:15, v/v) were used as the mobile phase at a flow rate of 1·0 mL min^{-1}. Acetate (0·1%) was used at a flow rate of 0·6 mL min^{-1} as the mobile phase for 5-fluorouracil. The mobile phases were filtered by passing 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.

Phenol red was assayed spectrophotometrically at 550 nm under alkaline conditions by diluting with 1 M NaOH.

Results and discussion

The penetration profiles of phenol red after application with MP and/or LP are shown in Fig. 1. Rat full-thickness skin and isopropyl myristate were used as models of a diffusion membrane and organic formulation. Phenol red is an unabsorbable hydrophilic dye. The suspension was used as a formulation to determine the maximum penetration of penetrant. Phenol red was not detected in the receptor phase after application alone. LP and MP enhanced penetration of the dye, the profile of which showed a lag phase followed by a linear rise. The lag time and

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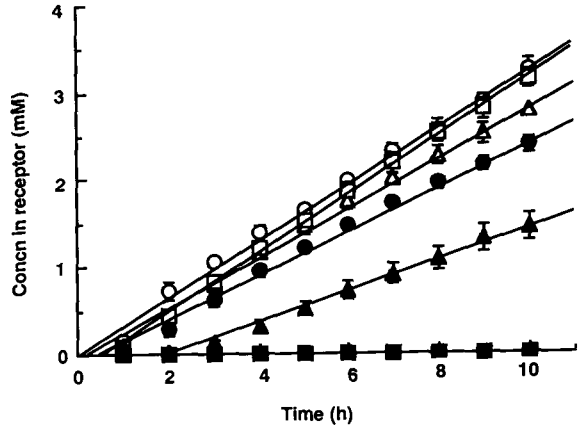

FIG. 1. Penetration-time profile of phenol red after application of phenol red with combined vehicle of MP and LP. Application concentration (mmol mL^{-1}) of MP and LP. 2:2 (○), 1:2 (△), 0·5:2 (□), 0·1:2 (●), 0:2 (▲), 2:0 (■). Each point represents the mean ± s.e.m. of at least three experiments.

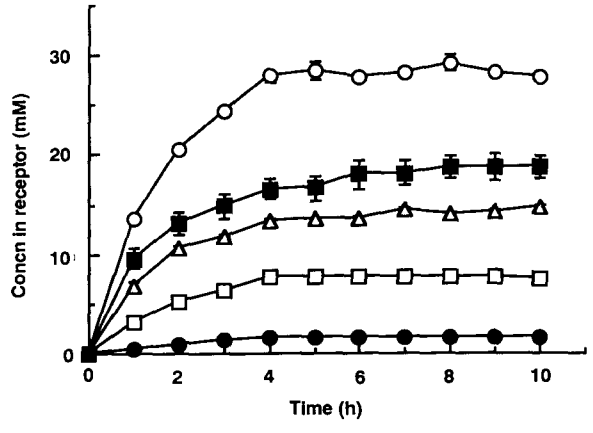

FIG. 2. Penetration-time profile of MP after application of phenol red with combined vehicle of MP and LP. Application concentration (mmol mL^{-1}) of MP and LP. 2:2 (○), 1:2 (△), 0·5:2 (□), 0·1:2 (●), 2:0 (■). Each point represents the mean ± s.e.m. of at least three experiments.

Table 1. Percutaneous penetration characteristics of phenol red after application of phenol red with combined vehicle of MP and LP.

Concn[a] (mmol mL^{-1})		n[b]	Lag time[c] (h)	Flux[c] (μmol cm^{-2} h^{-1})
MP	LP			
0	0	6	—[d]	—[d]
2	0	10	0·62 ± 0·16	0·02 ± 0·01
2	2	3	0·10 ± 0·04	2·39 ± 0·08
1	2	3	0·29 ± 0·03	2·18 ± 0·11
0·5	2	3	0·58 ± 0·11	2·44 ± 0·08
0·1	2	3	0·55 ± 0·16	1·88 ± 0·05
0	2	6	2·14 ± 0·09	1·34 ± 0·06

[a] Concentration of enhancer in the formulation. [b] Number of trials. [c] Lag time and flux were calculated graphically from the result of phenol red penetration. Each value represents the mean ± s.e.m. [d] Not detected.

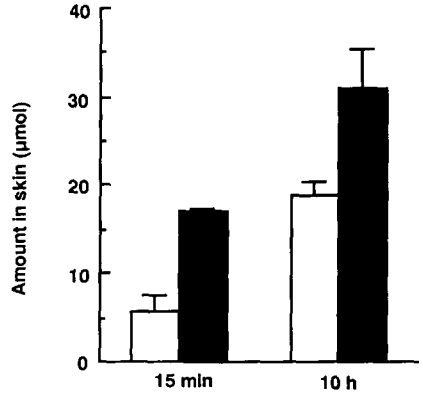

FIG. 3. Accumulation of LP in the skin at 15 min and 10 h after application of phenol red with LP (□) or combined vehicle of MP and LP (■). Each bar represents the mean ± s.e.m. of at least three experiments.

penetration flux of phenol red were estimated graphically and are summarized in Table 1. MP showed only low promoting activity. LP had a high enhancing effect on dye penetration, but there was a long lag time before the constant enhancing effect was attained. The combined vehicle of MP and LP showed a potent effect without a long lag time.

Berner et al (1989a, b) indicated the importance of an organic solvent flux from their results in which they found that the flux of nitroglycerin in aqueous ethanol solutions across skin was linear with the ethanol flux. Wotton et al (1985) also reported that propylene glycol as solvent was necessary for maximal enhancement on the transport of metronidazole, while Azone enhanced the penetration of propylene glycol. The penetration of MP is shown in Fig. 2. However, LP was not observed in the receptor phase (data not shown) but enhanced the penetration of MP. A reduction of the concentration of MP in the formulation decreased its penetration. In the present study, the combined vehicle showed a high enhancing effect regardless of the penetration of MP, suggesting that the high enhancing effect was not related to the penetration of MP.

Skin accumulations of phenol red, MP and LP at 10 h after their coapplications were determined and are summarized in Table 2. The skin accumulations of phenol red and MP increased with increase of their degree of penetration. LP showed a high accumulation in the skin in spite of not appearing in the receptor phase. MP enhanced the accumulation of LP in the skin.

Thus, the enhancers enhanced each other's penetration into the skin, LP enhancing the penetration of MP and MP enhancing the skin accumulation of LP. Therefore the combined vehicle seems to act synergistically, rather than additively, on penetrant penetration. Sheth et al (1986) reported that Azone and propylene glycol acted synergistically on the penetration of an antiviral compound. However, we found that the enhancing effect of MP on the penetration of phenol red was negligible with the combined vehicle because LP showed a much higher enhancing effect than MP. As shown in Fig. 3, LP showed a high skin accumulation at 15 min after application with MP, which is approximately equal to that at 10 h after application of LP alone. These results indicated that MP promoted the rapid penetration of LP into the skin and also potentiated the enhancing effect of LP and shortened the lag time.

5-Fluorouracil has been used for the treatment of carcinoma of the breast and gastrointestinal tract. Its topical application has also proved to be valuable for various diseases including epithelial neoplasms and psoriasis (Klein et al 1970; Tsuji & Sugai 1972). However, 5-fluorouracil is a hydrophilic compound, like phenol red, which cannot penetrate the hydrophobic skin barrier efficiently. In the previous report (Sasaki et al 1990), LP enhanced the penetration of 5-fluorouracil (7·48 ± 0·28 μmol cm^{-2} h^{-1}) much more than MP (0·59 ± 0·09 μmol cm^{-2} h^{-1}). However, LP has a longer lag time (2·81 ± 0·14 h) than MP (0·69 ± 0·05 h). Morimoto et al (1986) also reported the high enhancing effect of Azone with a long lag time for penetration of 5-fluorouracil. A combined vehicle of MP and LP showed a high

Table 2. Skin accumulation of phenol red and pyrrolidone derivatives at 10 h after application of phenol red with the combined vehicle of MP and LP.

Concn[a] (mmol mL^{-1})		n[b]	Amount[c] (μmol)		
MP	LP		Phenol red	MP	LP
0	0	6	—[d]	—[d]	—[d]
2	0	8	0·58 ± 0·05	9·46 ± 1·26	—[d]
2	2	3	18·07 ± 2·75	12·74 ± 1·69	30·98 ± 4·41
1	2	3	17·80 ± 1·97	11·36 ± 1·56	58·65 ± 13·64
0·5	2	3	16·98 ± 0·92	4·77 ± 0·23	36·88 ± 1·79
0·1	2	3	14·64 ± 1·15	1·35 ± 0·41	20·48 ± 4·50
0	2	3	8·92 ± 0·63	—[d]	18·77 ± 1·53

[a] Concentration of enhancer in the formulation. [b] Number of trials. [c] Amount of phenol red, MP and LP in the skin at 10 h after application. Each value represents the mean ± s.e.m. [d] Not detected.

Table 3. Enhancing effect of combined vehicle of MP and LP on the penetration of 5-fluorouracil.

Formulation[a]	n[b]	Lag time[c] (h)	Flux[c] (μmol cm^{-2} h^{-1})	Amount[d] (μmol)
Control	3	0·57 ± 0·07	0·057 ± 0·010	0·57 ± 0·07
Combined vehicle	4	0·09 ± 0·04	5·85 ± 0·19	18·52 ± 6·07

[a] Control: drug suspension in isopropyl myristate. Combined vehicle: drug suspension in isopropyl myristate containing MP (2 mmol mL^{-1}) and LP (2 mmol mL^{-1}). [b] Number of trials. [c] Lag time and flux were calculated graphically from the result of phenol red penetration. Each value represents the mean ± s.e.m. [d] Amount of 5-fluorouracil in the skin at 10 h after application. Each value represents the mean ± s.e.m.

enhancing effect on the penetration of 5-fluorouracil without a long lag time, as shown in Table 3. The combined vehicle also increased the accumulation of penetrant in the skin.

We conclude that the combined vehicle of two enhancers is useful for developing the transdermal drug delivery system, although further work is required to prove, in more detail, the mechanism by which the promoters elicit their action.

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Glycyrrhetinic acid derivatives: anti-nociceptive activity of deoxoglycyrrhetol dihemiphthalate and the related compounds

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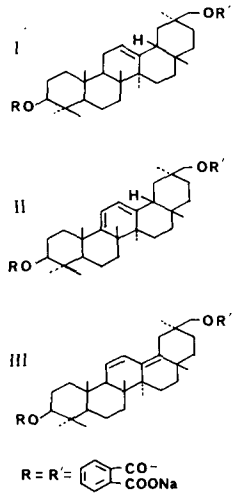
Abstract—The possible inhibitory effect of deoxoglycyrrhetol dihemiphthalate (I) and the related compounds (18β-olean-9(11),12-diene-3β,30-diol) (II) and (olean-11,13(18)-diene-3β,30-diol) III derived from glycyrrhetinic acid has been examined on acetic acid-induced writhing in mice. The compounds inhibited writhing dose-dependently. Their ED50 values were 14, 31 and 22 mg kg^{-1} for I, II, and III, respectively. The compounds like aspirin, also significantly suppressed PGE_2 production in peritoneal fluid together with the writhing response. The results suggests that the analgesic effect of deoxoglycyrrhetol dihemiphthalate and the related compounds is partially due to inhibition of PGE_2 production.

Glycyrrhetinic acid is the aglycone of glycyrrhizin, a pharmacologically active saponin of liquorice (Glycyrrhiza spp.) root. Glycyrrhetinic acid has also been found to be anti-inflammatory (Finney & Somers 1958; Capasso et al 1983), to antagonize tumour promotion (Nishino et al 1986) and to inhibit the growth of mouse melanoma (Abe et al 1987).



Deoxoglycyrrhetol (18β-olean-12-ene-3β, 30-diol) dihemiphthalate (I) and the related compounds (18β-olean-9(11),12-diene-3β,30-diol) (II) and (olean-11,13(18)-diene-3β,30-diol) (III) derived from glycyrrhetinic acid (Shibata et al 1987) were shown to inhibit lipoxygenase and cyclo-oxygenase activities in a cell-free system using mastocytoma cells (Inoue et al 1986) and mouse ear oedema induced by arachidonic acid (Inoue et al 1988) and tetradecanoyl phorbol acetate (Inoue et al 1989). In addition, these compounds were previously reported to strongly suppress the writhing response and vascular permeability induced by acetic acid, whose mechanism, however, has not so far been elucidated (Inoue et al 1987).

The present paper concerns a mode of action of deoxoglycyrrhetol dihemiphthalate and the related compounds on the analgesic effect.

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Materials and methods

Assay for acetic acid-induced writhing response. Male ddY mice, 6 weeks old (Shizuoka Laboratory Animal Center, Japan), were acclimatized under the standard conditions for one week before use, with free access to food and water. Dihemiphthalate compounds (I-III) were prepared according to Shibata et al (1987), and aspirin (Nakarai Chemical Co., Japan) was used as a reference. Test compounds were dissolved in 0·9% NaCl (saline) containing 1% Tween 80 (polyoxyethylene sorbitan monooleate, Tokyo Kasei Chemical Industry, Japan) and given orally 45 min before intraperitoneal injection (10 mL kg^{-1}) of 0·7% acetic acid. Control mice received vehicle only. The number of writhings of each mouse was counted during the first 30 min after injection of acetic acid. Statistical significance of the differences between groups was determined using the unpaired Student's *t*-test.

Assay of PGE_2 production in peritoneal fluid. The mice used in the writhing test were immediately killed 20 min after irritant treatment and injected with 5 mL saline intraperitoneally. The fluid collected from the peritoneal cavity was then centrifuged at 4500 *g* for 15 min at 4°C. Measurement of PGE_2 in the supernatant was based on the method of Kawano et al (1987), the PGE_2 was absorbed by ODS resin, eluted with ethyl acetate, and measured by radioimmunoassay (New England Nuclear, USA). The recovery was 98%. The cross-reactivity of anti-PGE_2 serum was as follows: 100% for PGE_2, 3·7% for PGE_1, 0·4% for PGA_2, 0·03% for PGF_1, 0·02% for TXB_2.

Results and discussion

Following the previous study (Inoue et al 1987), the present experiment showed that deoxoglycyrrhetol dihemiphthalate (I) and compounds (II and III) dose dependently inhibited the writhing response induced by 0·7% acetic acid (10 mL kg^{-1} i.p.) (Fig. 1). The ED50 values were 14, 31 and 22 mg kg^{-1} p.o. for I, II and III, respectively. Glycyrrhetinic acid, the parent compound had little effect at less than 200 mg p.o.

The effect of compounds I and II on acetic acid-induced PGE_2 production in peritoneal fluid was further examined with radioimmunoassay. As shown in Table 1, these compounds significantly inhibited PGE_2 production ($P < 0.05$) at a dose of 25 mg kg^{-1} compared with the control PGE_2 values (vehicle only