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Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use

Chomchan Amnuaikit[a,b], Itsue Ikeuchi[a], Ken-ichi Ogawara[a], Kazutaka Higaki[a], Toshikiro Kimura[a,*]

[a] *Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700-8530, Japan*
[b] *Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90112, Thailand*

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Abstract

To develop the suitable film formulations of propranolol hydrochloride (PPL) containing enhancers for transdermal use, polymeric film formulations were prepared by employing ethyl cellulose (EC) and polyvinyl pyrrolidone (PVP) as a film former, and dibutyl phthalate (DBP) as a plasticizer. Terpenes such as menthol and cineole, and propylene glycol (PG) were also employed as a chemical enhancer to improve the skin penetration of PPL. The film preparations were characterized in physical properties such as uniformity of drug content, thickness and moisture uptake capacity. Release and skin permeation kinetics of PPL from film preparations were examined in the in vitro studies using a Franz-type diffusion cell. The uniformity of drug content was evidenced by the low S.D. values for each film preparation. The moisture uptake capacity and drug release rate increased with the increase of PVP in each preparation. Enhancers examined in the present study also increased the moisture uptake capacity and release rate of PPL from the film preparations. Increasing the concentration of PPL from 1 to 2 mg/cm^2 in the film enhanced the release rate of PPL, while no effect of enhancer concentrations on the release rate from the film preparations was observed. In vitro skin permeation study showed that cineole was the most promising enhancer among the enhancers examined in the present study and suggested that the suitable compositions of film preparation would be EC:PVP:PPL = 6:3:4 with 10% (w/w) cineole and 7:2:4 with 10% (w/w) PG and cineole, which provided high skin permeation rates at 93.81 ± 11.56 and 54.51 ± 0.52 μg/cm^2/h, respectively.

Keywords: Propranolol hydrochloride; Terpene; Transdermal absorption; Polymeric film; Cineole; Propylene glycol

1. Introduction

Oral administration is one of the most convenient ways that are acceptable for patients, useful and suitable for some drugs that are not subjected to intestinal

* Corresponding author. Tel.: +81 86 251 7948; fax: +81 86 251 7926.
E-mail address: kimura@pheasant.pharm.okayama-u.ac.jp (T. Kimura).

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and/or hepatic first-pass metabolism (Kimura and Higaki, 2002). However, there are several disadvantages that should be overcome for achieving the efficient drug therapy as follows: the intestinal and/or hepatic first-pass elimination, high variance in bioavailability due to variable condition of gastrointestinal tract, difficulty in long-term and rate-regulated absorption and impossibility of arbitrary drug input and its interruption (Higaki et al., 2003). Transdermal route is one of the potent alternative routes that can improve undesirable characteristics of oral administration. Particularly, as propranolol, a β-blocker, has a short biological half-life and is subjected to extensive hepatic first-pass metabolism (Walle et al., 1979; Sawamoto et al., 1997), propranolol must be a potential candidate for the transdermal use. Recently, development of transdermal drug delivery systems (TDDS) has been focused on the formulation that can achieve the desirable constant rate of drug penetration into the systemic circulation, especially by employing several polymers as matrices or membranes controlling the release of drugs (Kou, 2000). On the other hand, the impermeability of human skin is still a fundamental problem to be overcome for the therapeutic use of TDDS (Barry, 2001a). Although many approaches have been proposed to overcome the stratum corneum, a main barrier for transdermal drug absorption (Higaki et al., 2003), chemical approaches such as a utilization of chemical enhancers might be only applicable to patch preparations. Among many enhancers examined, terpenes have been extensively investigated for their clinical use as an penetration enhancer and suggested to increase drug diffusivity in the skin by disrupting the intercellular lipid packing in the horny layer (Vaddi et al., 2002; Higaki et al., 2003). Considering the balance between efficiency and toxicity, several terpenes may be promising chemical enhancers for clinical use (Kitahara et al., 1993; Higaki et al., 2003). In the present study, we tried to develop a suitable film preparation of propranolol hydrochloride (PPL) by employing ethyl cellulose (EC) and polyvinyl pyrrolidone (PVP) as a film former, and dibutyl phthalate (DBP) as a plasticizer. Furthermore, in order to improve the penetration of PPL, terpenes such as menthol and cineole, and propylene glycol (PG) were employed as a chemical enhancer. Release and permeation profiles of PPL from film preparations were examined in the in vitro studies using a Franz-type diffusion cell.

2. Materials and methods

2.1. Materials

EC (with an ethoxy content 47.5–53.5% by weight and a viscosity of 9–11 cps in a 5% (w/w), 80:20 toluene/ethanol solution at 25 °C, Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), PVP K30, DBP, chloroform (HPLC grade) and cineole were obtained from Nacalai Tesque (Kyoto, Japan). PPL, PG and menthol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals obtained commercially were of a reagent grade.

2.2. Animals

Male Wistar rats (Japan SLC, Hamamatsu, Japan), maintained at 25 °C and 55% humidity were allowed free access to standard laboratory chow (Clea Japan, Tokyo) and water prior to the experiments. Rats weighing 150–200 g were randomly assigned to each experimental group. Our investigations were performed after approval from the local ethical committee at Okayama University and in accordance with ‘Interdisciplinary Principles and Guidelines of the Use of Animals in Research’.

2.3. Preparation of film formulations containing PPL

Films composed of different ratios of EC, PVP, enhancers and PPL were prepared by a method reported previously (Kurosaki et al., 1988). All the ingredients were weighed in requisite ratio and they were then dissolved in 25 ml of chloroform. DBP was incorporated at a concentration of 30% (w/w) of dry weight of polymers as a plasticizer. An enhancer was dissolved at a concentration of 5% or 10% (w/w) of total dry weight of EC, PVP and DBP. The resultant chloroform solutions were poured into a Teflon tray, and were dried at 45 °C for 12 h.

2.4. Film thickness

The thickness of films was measured at three different places using a micrometer (Mitutoyo Co., Kanagawa, Japan) and mean values were calculated.

2.5. Determination of drug content in the film

The uniformity of drug distribution was evaluated by determining drug content at different places of the film by a spectrophotometric method (United States Pharmacopeia, 1995). A known weight of film was dissolved and diluted subsequently with chloroform, and the concentration of PPL was spectrophotometrically measured at 290 nm (Shimadzu UV-260, Shimadzu, Kyoto) against the blank chloroform solution containing the same amount of polymer and plasticizer without drug.

2.6. Moisture uptake study

After films, of which the size is 1 cm × 1 cm in a square, were put in a desiccator with silica gel for 24 h and weighed (W_s), the films were transferred to another desiccator containing saturated NaCl solution (relative humidity 75%) at 25 °C. After equilibrium was attained, the films were taken out and weighed (W_m). Moisture uptake capacity was calculated according to the following equation:

$$\text{Moisture uptake capacity (\%)} = \frac{W_m - W_s}{W_s} \times 100$$

2.7. In vitro drug release study

The release of drug from film preparations was examined using a modified Franz-type diffusion cell. The films cut in a circle shape were put on a glass filter paper placed on the receptor cell, of which the effective area for diffusion was 3.14 cm^2. The receptor compartment was filled with 18 ml of isotonic phosphate buffer solution (PBS). The diffusion cell was thermoregulated with a water jacket at 37 °C and the receptor compartment was stirred with a magnetic stirrer. Samples (2 ml) were withdrawn at 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h. An equal volume of fresh PBS was immediately added to the receptor cell after each sampling. The concentration of PPL was spectrophotometrically determined at 289 nm (Shimadzu UV-260).

2.8. In vitro skin permeation study

Abdominal hair was removed using 7% thioglycolic acid gel 2 days before performing the isolation of rat abdominal skin (Higaki et al., 2002). The shaved abdominal skin was carefully excised from Wistar rats as described previously and the subcutaneous tissue and adipose tissue were carefully removed (Yagi et al., 1998). The obtained skin preparations were mounted in a Franz diffusion cell. The film preparation was placed on the skin and fixed and covered by the upper compartment of a Frantz-type cell. Experimental condition of diffusion cell and sampling procedure were the same as in the case of drug release study. Concentration of PPL in PBS of the receptor compartment was determined by HPLC system, which consists of a model LC-6A HPLC pump (Shimadzu) and a UV detector (SPD-6A, Shimadzu) set at 289 nm. Analytical column was Inertsil ODS-3 ($5C_{18}$, 250 mm × 4.6 mm i.d., GL Sciences, Tokyo). The mobile phase (CH_3CN:20 mM NH_4Cl:0.05% phosphoric acid = 1:1:1 (v/v)) was delivered at 1 ml/min. The coefficient of variation (CV) for standard curves ranged from 0.06 to 18.7% and the squared correlation coefficient was over 0.9981. The cumulative amount of drug permeated was plotted against time. The flux values were calculated from the linear portions of the plots.

2.9. Statistical analysis

Results are expressed as the mean ± S.D. of at least three experiments. Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Dunnet's method or Student's *t*-test.

3. Results

Polymeric film formulations containing various ratios of EC:PVP, loaded with 1 mg/cm^2 PPL, were prepared and their physicochemical properties such as uniformity of drug content, thickness and moisture uptake capacity were examined (Table 1). Estimation of drug content at different places on each film indicated that PPL was distributed uniformly throughout the films. There was no significant effect of film ingredients on the thickness of films. On the other hand, the increase in the ratio of PVP significantly enhanced the moisture uptake, which was confirmed by the significant relationship between the

Table 1
Physicochemical properties of film formulations of PPL

Enhancers	EC:PVP:PPL	Drug content (%)	Thickness (mm)	Moisture uptake capacity (%)	r^2[a]
No enhancer	9:0:2	87.7 ± 0.5	0.060 ± 0.010	1.8 ± 0.0	
	8:1:2	97.8 ± 2.2	0.050 ± 0.010	2.1 ± 0.0	
	7:2:2	102.5 ± 0.7	0.060 ± 0.010	3.7 ± 0.0	0.9469
	6:3:2	93.1 ± 0.7	0.060 ± 0.010	4.5 ± 0.0	
	5:4:2	102.8 ± 0.2	0.057 ± 0.015	6.6 ± 0.1	($p < 0.01$)
Propylene glycol	9:0:2	83.1 ± 0.3	0.057 ± 0.006	1.8 ± 0.1	
	8:1:2	91.6 ± 1.2	0.050 ± 0.010	2.9 ± 0.0	
	7:2:2	102.7 ± 0.5	0.057 ± 0.012	5.9 ± 0.1	0.9499
	6:3:2	95.3 ± 1.5	0.057 ± 0.006	6.2 ± 0.1	
	5:4:2	89.7 ± 0.5	0.063 ± 0.006	8.0 ± 0.1	($p < 0.005$)
Menthol	9:0:2	85.5 ± 1.0	0.060 ± 0.017	2.2 ± 0.1	
	8:1:2	90.9 ± 0.6	0.060 ± 0.010	2.8 ± 0.1	
	7:2:2	101.4 ± 1.3	0.050 ± 0.010	5.4 ± 0.0	0.9661
	6:3:2	92.3 ± 0.7	0.063 ± 0.012	7.2 ± 0.0	
	5:4:2	92.2 ± 0.3	0.050 ± 0.010	8.1 ± 0.1	($p < 0.005$)
Cineole	9:0:2	90.1 ± 1.5	0.057 ± 0.006	2.4 ± 0.0	
	8:1:2	95.8 ± 0.7	0.060 ± 0.010	3.2 ± 0.1	
	7:2:2	94.5 ± 0.8	0.060 ± 0.010	6.9 ± 0.1	0.9095
	6:3:2	98.5 ± 2.6	0.050 ± 0.017	7.4 ± 0.1	
	5:4:2	101.6 ± 1.3	0.060 ± 0.010	8.4 ± 0.1	($p < 0.02$)
Propylene glycol and menthol	9:0:2	91.4 ± 1.1	0.060 ± 0.010	2.1 ± 0.1	
	8:1:2	102.9 ± 0.0	0.053 ± 0.012	3.0 ± 0.0	
	7:2:2	99.5 ± 1.3	0.060 ± 0.010	7.1 ± 0.1	0.8591
	6:3:2	101.9 ± 1.3	0.060 ± 0.010	7.1 ± 0.1	
	5:4:2	97.7 ± 1.0	0.050 ± 0.010	7.9 ± 0.1	($p < 0.05$)
Propylene glycol and cineole	9:0:2	97.4 ± 2.5	0.063 ± 0.012	3.1 ± 0.1	
	8:1:2	91.7 ± 1.2	0.057 ± 0.006	4.0 ± 0.1	
	7:2:2	93.5 ± 0.9	0.050 ± 0.010	7.0 ± 0.1	0.9210
	6:3:2	94.3 ± 0.1	0.053 ± 0.006	7.6 ± 0.1	
	5:4:2	96.5 ± 0.7	0.053 ± 0.006	8.2 ± 0.0	($p < 0.01$)

Results are expressed as the mean ± S.D. of three experiments. EC, PVP and PPL mean ethyl cellulose, polyvinyl pyrrolidone and propranolol hydrochloride, respectively. Loaded amount of PPL in each film was 1 mg. Concentration of each enhancer was 5% (w/w).

[a] A square of correlation coefficient between the moisture uptake % and the ratio of PVP in each film.

Table 2
Higuchi's rate constant of PPL (1 mg/cm^2) for film formulations calculated by following Higuchi's model

EC:PVP:PPL	No enhancer	PG	Menthol	Cineole	PG + menthol	PG + cineole
9:0:2	35.8 ± 1.0	51.0 ± 3.2[b]	49.6 ± 8.5[b]	59.0 ± 6.3[b]	50.8 ± 5.6[b]	54.3 ± 6.8[b]
8:1:2	53.0 ± 2.7	80.1 ± 6.1[b]	61.6 ± 2.8	84.3 ± 17.4[b]	73.6 ± 11.8	69.7 ± 8.1
7:2:2	61.0 ± 6.0	167.4 ± 13.4[a,b]	91.4 ± 42.6	207.1 ± 13.8[a,b]	93.0 ± 14.8[a]	105.2 ± 6.3[a]
6:3:2	159.5 ± 23.2[a]	341.2 ± 7.9[a,b]	243.8 ± 21.9[a,b]	245.9 ± 13.8[a,b]	297.7 ± 18.5[a,b]	292.3 ± 22.3[a,b]
5:4:2	212.8 ± 20.9[a]	417.0 ± 32.1[a,b]	288.7 ± 18.4[a,b]	314.1 ± 54.6[a,b]	332.7 ± 19.5[a,b]	325.7 ± 29.9[a,b]

Results are expressed as the mean ± S.D. of three experiments. EC, PVP, PPL and PG mean ethyl cellulose, polyvinyl pyrrolidone, propranolol hydrochloride and propylene glycol, respectively. Unit of Higuchi's rate constant of PPL is μg/cm^2/h$^{1/2}$. Concentration of each enhancer was 5% (w/w).

[a] $p < 0.05$ when compared with ratio of EC:PVP:PPL 9:0:2 as the control in no enhancer and each enhancer.

[b] $p < 0.05$ when compared with no enhancer as the control in corresponding ratio of EC:PVP:PPL.

moisture uptake and the ratio of PVP in films. Each enhancer (5% w/w) increased the moisture uptake capacity, but cineol and the combination of PG with cineole tended to give higher capacity than other film preparations.

Release of PPL from film preparations was examined in an in vitro study using a Frantz-type diffusion cell (Fig. 1 and Table 2). As the regression analysis of obtained results for three kinetic models such as zero order, first order and Higuchi's model showed that Higuchi's model gave the highest value of r^2 with significant difference ($p < 0.05$), Higuchi's model, where the cumulative amount of released drug per unit area is proportional to the square root of time, is the most suitable model to describe the release kinetics of PPL from the film preparations examined in the present study. Higuchi's rate constants calculated are summarized in Table 2. Fig. 1 shows the release profile of PPL from film preparations containing no enhancer, 5% (w/w) cineole or 5% (w/w) PG and cineole as a typical example. The release rate of PPL from film preparations tended to increase as PVP fraction in the film increased (Fig. 1 and Table 2). Furthermore, the addition of an enhancer or enhancers also promoted the release of drug from the film preparations more (Fig. 1 and Table 2).

In vitro skin permeation studies were performed to evaluate transdermal absorption of PPL from these film preparations. Fig. 2 depicts the permeation profile of PPL from film preparations containing 5% (w/w) cineole, which provided the highest permeation rate among enhancers examined in the present study. Table 3 shows the permeation rates of PPL for all the film preparations. Results show that there is an optimal ratio of film formers for each enhancer to show the highest permeation rate of PPL. The film (EC:PVP:PPL = 6:3:2) containing 5% (w/w) cineole gave the highest permeation rate among the film preparations containing 5% (w/w) enhancer or enhancers.

To improve the skin permeation of PPL from film preparations further, the loading concentrations of PPL and enhancers were increased up to 2 mg/cm^2 and 10%, respectively. The ratio of film formers that gave the highest permeation rate of PPL for each enhancer was selected based on the results shown in Table 3. Because of recrystallization, 2 mg/cm^2 was almost a maximal dosing concentration of PPL in the film preparations. Physicochemical properties for these

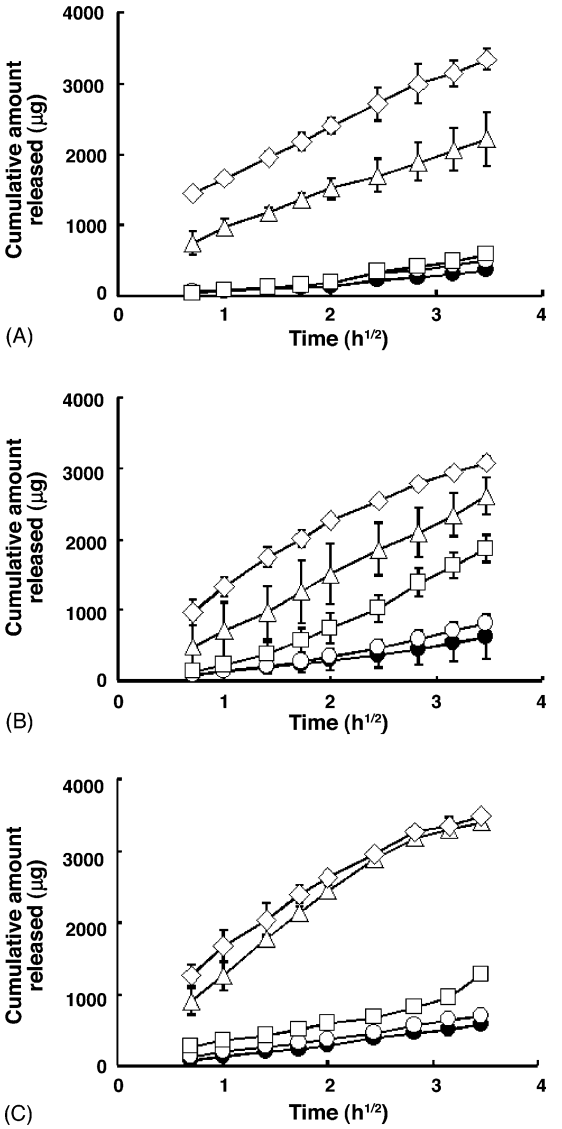

Fig. 1. Effect of ratio of EC and PVP on release profile of PPL from film preparations without any enhancer (A), film preparations containing 5% (w/w) cineole (B) and film preparations containing 5% (w/w) PG + cineole (C). PPL was contained in the films at 1 mg/cm^2. Cumulative PPL amount released was plotted against the square root of time, because Higuchi's model was found to be the suitable model for describing the release profile of PPL. Results are expressed as the mean with the bars showing S.D. values of three and more different experiments. Keys: EC:PVP = 9:0 (●), 8:1 (◯), 7:2 (□), 6:3 (△) and 5:4 (◊).

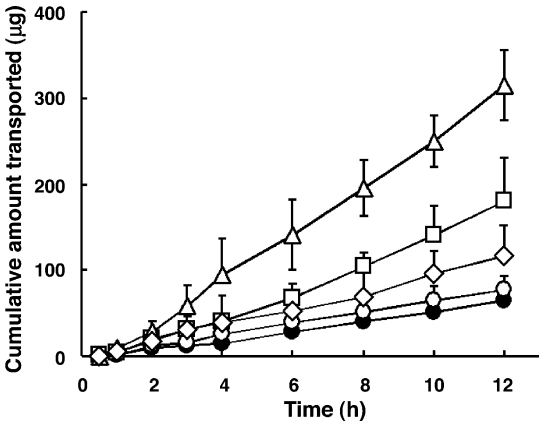

Fig. 2. Effect of ratio of EC and PVP on skin permeation of PPL through rat skin from films containing 5% (w/w) cineole. PPL was contained in the films at 1 mg/cm^2. Results are expressed as the mean with the bars showing S.D. values of three different experiments. Keys: EC:PVP = 9:0 (●), 8:1 (◯), 7:2 (□), 6:3 (△) and 5:4 (◊).

film preparations are summarized in Table 4. Loading of 2 mg/cm^2 PPL tended to thicken films and significantly enhanced the capacity of moisture uptake. The increase of enhancer concentration in the film tended to increase the moisture uptake capacity more. Fig. 3A shows the effect of PPL concentration on the release kinetics, indicating that Higuchi's rate constant of PPL was increased around three times from 212.79 ± 20.91 to 709.81 ± 53.79 μg/cm^2/h$^{1/2}$, as the concentration of PPL increased twice. On the other hand, there was no effect of cineole concentration on the release profile of PPL (Fig. 3B). Higuchi's rate constants of PPL for the preparations containing cineole are as follows: the film containing 5% (w/w) cineole, 693.38 ± 132.74 μg/cm^2/h$^{1/2}$; 10% (w/w) cineole, 672.02 ± 27.17 μg/cm^2/h$^{1/2}$.

Fig. 4(A) shows the in vitro skin permeation profiles for film preparations containing 2 mg/cm^2 PPL and 5% (w/w) enhancers. Fig. 4(B) shows the effect of 10% (w/w) enhancer on the skin permeation of PPL. The permeation rates for all the preparations are summarized in Table 5. Skin permeation of PPL was significantly enhanced by increasing the loading concentration of PPL in every preparation except for one containing PG. The highest permeation rate was observed in the film containing cineole, which was followed by the film containing PG and cineole. Increasing the concentration of enhancers significantly enhanced the skin permeation of PPL only for the two preparations containing cineole or PG and cineole, and the film preparation containing 10% (w/w) cineole provided the highest permeation rate of PPL among the preparations examined in the present study, around 8.5-fold of the film preparation (EC:PVP:PPL = 5:4:4) without any enhancer.

4. Discussion

TDDS is one of the promising alternatives to oral dosage forms especially for drugs that are subjected to the first-pass elimination such as PPL. To optimize the release of drug from the TDDS as close to a desired profile as possible for the long-time period of operation, much attention has recently been focused on the development of a film preparation composed of several polymers (Jain et al., 1996; Rama Rao et al., 2000; Shin et al., 2002). In the present study, we tried to prepare polymeric film formulations of PPL that would make it possible for PPL to penetrate the skin at a high and constant rate by employing several terpenes such as menthol and cineole.

Table 3
Skin permeation rate of PPL from film preparations containing 1 mg/cm^2 PPL in an in-vitro transport study

EC:PVP:PPL	No enhancer	PG	Menthol	Cineole	PG + menthol	PG + cineole
9:0:2	4.3 ± 1.8	6.9 ± 1.4	12.9 ± 3.5[b]	5.7 ± 0.2	3.2 ± 0.9	3.2 ± 0.3
8:1:2	5.1 ± 0.9	7.7 ± 0.8	6.3 ± 0.5[a]	6.7 ± 1.4	8.8 ± 1.9[b]	6.8 ± 1.8
7:2:2	3.6 ± 0.3	8.3 ± 1.5	10.0 ± 1.0[b]	15.4 ± 4.2[a,b]	16.6 ± 4.7[a,b]	21.1 ± 0.7[a,b]
6:3:2	6.2 ± 1.9	13.5 ± 2.1[a,b]	6.8 ± 0.3[a]	27.4 ± 3.9[a,b]	6.8 ± 1.9	12.1 ± 5.5[a]
5:4:2	6.3 ± 1.9	9.8 ± 3.0	16.6 ± 2.4[b]	9.9 ± 3.0	8.3 ± 2.6	9.8 ± 1.9[a]

Results are expressed as the mean ± S.D. of three experiments. EC, PVP, PPL and PG mean ethyl cellulose, polyvinyl pyrrolidone, propranolol hydrochloride and propylene glycol, respectively. Unit of permeation rate of PPL is μg/cm^2/h. Concentration of each enhancer was 5% (w/w).

[a] $p < 0.05$ when compared with ratio of EC:PVP:PPL 9:0:2 as the control in no enhancer and each enhancer.

[b] $p < 0.05$ when compared with no enhancer as the control in corresponding ratio of EC:PVP:PPL.

Table 4
Physical properties of film formulations containing 2 mg/cm^2 PPL

Enhancers (%)	EC:PVP:PPL	Drug content (%)	Thickness (mm)	Moisture uptake capacity (%)
No enhancer	5:4:4	99.6 ± 0.7	0.070 ± 0.010	8.9 ± 0.3[a]
PG				
5	6:3:4	96.4 ± 1.4	0.073 ± 0.006	9.5 ± 0.4[b]
10	6:3:4	98.7 ± 0.4	0.080 ± 0.010	13.8 ± 0.8[c]
Menthol				
5	5:4:4	100.9 ± 0.6	0.070 ± 0.010	10.7 ± 1.5
10	5:4:4	96.7 ± 1.9	0.080 ± 0.010	13.4 ± 0.5
Cineole				
5	6:3:4	99.8 ± 0.8	0.063 ± 0.006	15.1 ± 0.9[b]
10	6:3:4	100.2 ± 0.9	0.073 ± 0.006	16.8 ± 0.3
PG + menthol				
5	7:2:4	96.3 ± 2.4	0.077 ± 0.006	11.1 ± 0.6[b]
10	7:2:4	97.9 ± 1.7	0.073 ± 0.012	14.1 ± 0.8[c]
PG + cineole				
5	7:2:4	99.3 ± 0.6	0.080 ± 0.010	10.4 ± 0.6[b]
10	7:2:4	99.8 ± 1.1	0.073 ± 0.012	14.0 ± 0.8[c]

Results are expressed as the mean ± S.D. of three experiments. EC, PVP, PPL and PG mean ethyl cellulose, polyvinyl pyrrolidone, propranolol hydrochloride and propylene glycol, respectively. Loaded amount of PPL in each film was 2 mg.

[a] $p < 0.05$ when compared with the same ratio of film former containing 1 mg/cm^2 PPL.

[b] $p < 0.05$ when compared with the same concentration of enhancer containing 1 mg/cm^2 PPL.

[c] $p < 0.05$ when compared with the same enhancer containing 5% (w/w) of enhancer.

The moisture uptake capacity increased with the increase of the PVP ratio in the film (Table 1), which can be supported by the previous reports (Rama Rao et al., 2000; Arora and Mukherjee, 2002). PVP might enhance the absorption of water vapor by converting the crystalline drug into amorphous state and making the molecules spaced further apart than in a crystal (Rama Rao et al., 2000). Each enhancer also increased

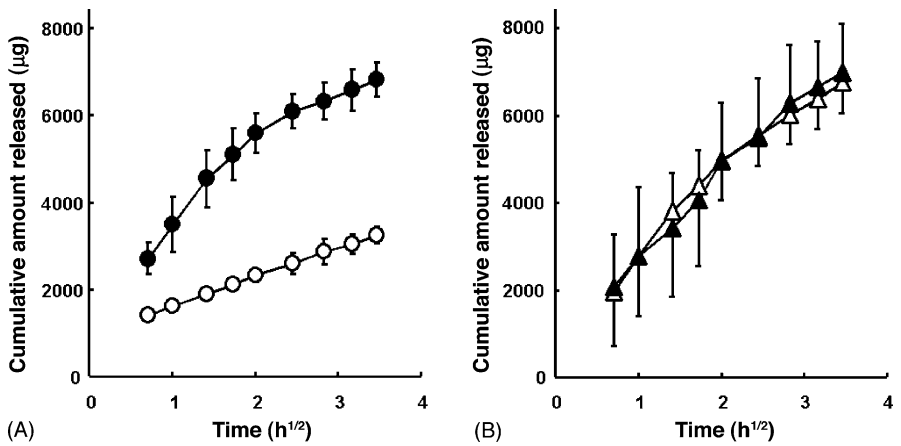

Fig. 3. Effect of PPL concentration (A) or cineole concentration (B) on release profile of PPL from film formulations. (A) PPL was contained at 1 mg/cm^2 (◯) or 2 mg/cm^2 (●). Ratio of film formers was EC:PVP = 5:4. (B) Cineole was contained at 5% (w/w) (△) or 10% (w/w) (▲) in the films containing 2 mg/cm^2 of PPL. Ratio of film formers was EC:PVP = 6:3. Cumulative PPL amount released was plotted against the square root of time, because Higuchi's model was found to be the suitable model for describing the release profile of PPL. Results are expressed as the mean with the bars showing S.D. values of three different experiments.

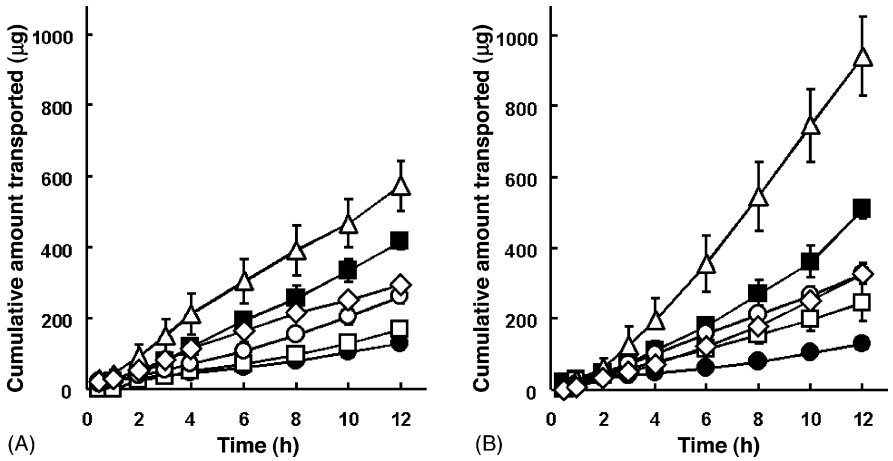

Fig. 4. Effect of enhancer concentration on permeation of PPL through rat skin from films. Each enhancer was contained at 5% (w/w) (A) or 10% (w/w) (B). Concentration of PPL was 2 mg/cm^2 for every preparation. Results are expressed as the mean with the bars showing S.D. values of three different experiments. Keys: no enhancer (●); PG (□); menthol (○); cineole (△); PG + menthol (◊) and PG + cineole (■).

the moisture uptake capacity (Table 1), for which the reason remains to be clarified. In the case of PG, however, it can absorb moisture from environment into the film where PG is contained because of its humectant ability (Barry, 1983).

The analysis of drug release profiles showed that Higuchi's model was the most suitable for describing the release kinetics of PPL from the films prepared in the present study (Figs. 1 and 3, Table 2), which means that the release of PPL from the film preparations is regulated by the diffusion of PPL within a matrix system (Siepmann and Peppas, 2001). The increase of Higuchi's rate constant with the increase of PVP content in the film (Fig. 1 and Table 1) can be explained by the leaching of PVP and pore formation (Rama Rao et al., 2000, 2003). Each enhancer and the combination of two enhancers also tended to promote the release of PPL from the film preparations (Fig. 1 and Table 2). Although the mechanisms for this effect remain to be clarified, the greater amount of water absorbed into the film by an enhancer or enhancers (Table 1) would contribute to the more rapid release of PPL from the films. Although the release rate of PPL did not increase in the film containing 10% (w/w) cineole compared with the

Table 5
Skin permeation rate of PPL from film preparations containing 2 mg/cm^2 PPL and 5% or 10% (w/w) enhancers in an in vitro transport study

Enhancers	EC:PVP:PPL	Permeation rate (μg/cm^2/h)			
		5% (w/w) Enhancer		10% (w/w) Enhancer	
		Mean ± S.D.	Ratio to 1 mg/cm^2-PPL Film	Mean ± S.D.	Ratio to 5%-enhancer film
No enhancer	5:4:4	11.3 ± 1.6[a]	1.79	–	–
PG	6:3:4	16.1 ± 1.2	1.19	20.6 ± 5.3	1.28
Menthol	5:4:4	24.0 ± 5.0[b]	1.45	28.2 ± 1.7[b]	1.18
Cineole	6:3:4	49.3 ± 5.5[b]	1.80	93.8 ± 11.6[b,c]	1.90
PG + menthol	7:2:4	24.2 ± 1.7[b]	1.46	30.6 ± 4.0[b]	1.27
PG + cineole	7:2:4	36.1 ± 3.0[b]	1.71	54.5 ± 0.5[b,c]	1.51

Results are expressed as the mean ± S.D. of three experiments. EC, PVP, PPL and PG mean ethyl cellulose, polyvinyl pyrrolidone, propranolol hydrochloride and propylene glycol, respectively.

[a] $p < 0.05$ when compared with the same ratio of film former containing 1 mg/cm^2 PPL.

[b] $p < 0.05$ when compared with no enhancer as the control.

[c] $p < 0.05$ when compared with the same enhancer containing 5% (w/w) of enhancer.

film containing 5% (w/w) cineole, this finding might be explained by no change of the moisture uptake capacity (Table 4). Increasing the concentration of PPL two times resulted in over three times greater rate of release from the films (Fig. 3(A)), but this finding agrees with another report (Rama Rao et al., 2000), where it was suggested that increasing the amount of drug dispersed in the film would increase the porosity, leading to the greater release rate than expected based on the increase in drug concentration.

Skin permeation studies showed that the film preparations containing cineole or menthol could enhance the skin penetration of PPL (Figs. 3 and 4). Especially, the film preparation containing 10% (w/w) cineole (EC:PVP:PPL = 6:3:4) or 10% (w/w) cineole and PG (EC:PVP:PPL = 7:2:4) provided the high permeation rate, 93.81 and 54.51 $\mu g/cm^2/h$, respectively (Table 5). These values are 3–6-fold of the films prepared by Rama Rao et al. (2003), suggesting that these film preparations can provide the good and long-lasting therapeutic effect. It is well known that the enhancing effect of chemical enhancers could be dependent on the physicochemical properties of drugs and the combination with vehicle or ingredients in preparations (Higaki et al., 2003), but terpenes can enhance both hydrophilic drugs including propranolol and lipophilic drugs such as testosterone (Williams and Barry, 1991a, 1991b; Kunta et al., 1997; Kaplun-Frischoff and Touitou, 1997; Vaddi et al., 2002). Especially, cineole and menthol improved the skin permeation of hydrophilic drugs better than other terpenes (Jain et al., 2002; Narishetty and Panchagnula, 2004). A proposed mechanism for terpenes to improve the skin permeation of drugs is mainly the increase in drug diffusivity in the skin (Williams and Barry, 1991a; Cornwell and Barry, 1994; Zhao and Singh, 1998; Vaddi et al., 2002) by modifying the intercellular packing, disrupting highly ordered structure of lipids (Barry, 1991). It has also been suggested that the molecular mechanism is attributed to the preferential hydrogen bonding of oxygen-containing monoterpenes with ceramide head groups thereby breaking the lateral/transverse hydrogen bond network of lipid bilayer (Jain et al., 2002; Narishetty and Panchagnula, 2004). Because of the short lag time (around 2 h) for the film preparation without any enhancer and the shape of cumulative transport profile, we could not find the significant change in the lag time for the films containing terpenes (data not shown). However, the transported amount of PPL clearly increased at early time periods, suggesting the increase in diffusivity of PPL in the skin.

Skin permeation studies also showed that there was an optimal ratio of film formers that gave the highest permeation rate of PPL for each enhancer (Table 3). The preparation giving the highest penetration rate was not necessarily coincided with the one giving the highest release rate of PPL (Table 2), meaning that the skin penetration of PPL is not regulated by drug release kinetics, but by the effect of enhancers on the skin permeation of PPL. Several factors such as the release kinetics of enhancers from the film and the interaction between enhancers and other ingredients in the film would influence the enhancing activity, and the effects of these factors might be changeable dependent on the ratio of film formers and enhancers. Cal et al. (2001) have investigated percutaneous penetration of five terpenes from the matrix type transdermal patches through the skin, and have indicated that eucalyptol (cineole) is one of the terpenes with the highest skin penetration rate. Narishetty and Panchagnula (2004) suggested that cineole would interact with lipid components of the stratum corneum more easily, because the boiling point of cineole was lower than other terpenes and the low boiling point is an indication of weak cohesiveness or self-association. These might explain the reason why cineole showed the highest enhancing effect on the skin permeation of PPL in the present study. In the case of the film preparations containing PG or cineole, the skin flux of PPL tended to be improved with the increase of PVP (Table 3), which might be attributed to an antinucleating effect of PVP that can convert the crystalline drug into amorphous state on the skin surface (Rama Rao et al., 2003). Compounds in amorphous state generally posses a high energy state with improved solubility and the enhancement of solubility of drug close to the skin surface increases thermodynamic activity that facilitates the permeation rate of drug through the skin (Rama Rao et al., 2003).

The effect of permeation enhancers often depends on their applied concentrations (Jain et al., 1996; Takayama et al., 1999; Narishetty and Panchagnula, 2004), and this is also the case with the preparations examined in the present study (Table 5). Only cineole and the combination of PG with cineole significantly increased the enhancing effect as the applied concentra-

tion increased, although the enhancing effect of other preparations was almost saturated.

The enhancing effect of PG itself was not so remarkable for PPL (Fig. 4, Tables 3 and 5), although it was reported that PG itself enhanced the skin permeation of several drugs (Polano and Ponec, 1976; Wotton et al., 1985; Irwin et al., 1990) without any alteration in the skin structure (Fang et al., 2003). The increase in solubilizing ability of the aqueous site in the stratum corneum is considered to be a main mechanism for PG to improve the skin permeation of drugs (Barry, 1991). However, the enhancing activity of PG itself is quite controversial (Asbill et al., 2000; Higaki et al., 2003), because PG also has several characteristics that can decrease the skin permeation of drugs as follows: (1) PG, classified as a humectant (Barry, 1983), has a dehydration effect on the skin, leading to the lower skin permeability, and the higher affinity of drugs for PG may contribute to the reduced drug permeation (Ross and Shah, 2000), (2) the solubilizing effect of PG might lead to the decrease in the chemical potential of drugs in the stratum corneum (Barry, 1991) and (3) as the viscosity of solution is one of the factors that affect the diffusion of drug (Barry, 1991; Hadgraft, 1999), the possible increase in the viscosity of the aqueous site in the stratum corneum by the penetration of PG might decrease the penetration rate of drugs. We confirmed that high viscosity caused by PG retarded the skin penetration of PPL from the aqueous solution (data not shown). On the other hand, it is also known that PG can enhance the skin penetration of several drugs by raising the concentrations of other enhancers in the horny layer (Wotton et al., 1985; Barry and Williams, 1989; Barry, 2001b; Gwak and Chun, 2002). However, PG showed a remarkable synergistic effect with neither cineole nor menthol and the results included both enhancing and retarding effects of PG on the skin permeation of PPL (Tables 3 and 5). No synergistic effect of PG with other enhancers has been found in the case of flurbiprofen as well (Fang et al., 2003). Decrease in partition of enhancers into the skin was suggested to be a possible reason for it, which might also be the case with our studies. The partition of cineole and menthol could also be dependent on the ratio of film formers, which can affect the affinity of enhancers to the film preparations (Okabe et al., 1990; Cheong and Choi, 2003). Furthermore, as PG has a negative effect on the skin penetration of drugs described above, the balance between positive and negative actions of PG might be important for preparations containing PG to provide a suitable enhancing effect.

Terpenes are also well known as enhancers causing no local toxicity or, if any, only low irritation of the skin (Asbill et al., 2000; Krishnaiah et al., 2003). Especially, cineole and menthol are categorized to the group of terpenes showing no change or very slight irritation (Kitahara et al., 1993). Even terpenes classified to the group showing definite irritation did not cause lasting erythema at all (Okabe et al., 1990), which might be explained by the reversibility of the disrupting action of terpenes (Narishetty and Panchagnula, 2004). Actually, no obvious change of the skin surface was observed for every film preparation examined in the present study. Therefore, terpenes such as cineole and menthol could be promising chemical enhancers for transdermal use, considering the balance between efficacy and toxicity (Higaki et al., 2003).

5. Conclusion

To avoid the extensive first-pass elimination and achieve the desirable penetration rate of PPL, we prepared the polymeric film formulations for transdermal use by employing EC and PVP as a film former, and DBP as a plasticizer. The film containing 10% (w/w) cineole (EC:PVP:PPL = 6:3:4) and 10% (w/w) PG and cineole (EC:PVP:PPL = 7:2:4) successfully improved the skin penetration of PPL. Although a remarkable synergistic effect of PG was not observed, these film preparations would be promising ones that can safely achieve the desirable systemic absorption of PPL.

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