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

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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

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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

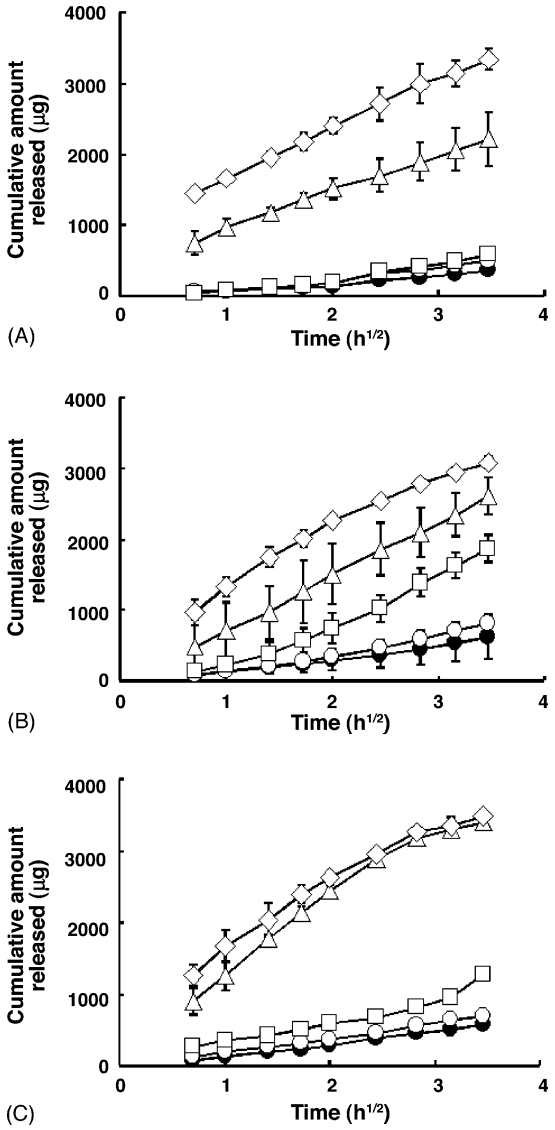

Fig. 1. Effect of ratio of EC and PVP on release profile of PPL from film preparations without any enhancer (A), film preparations containing 5% (w/w) cineole (B) and film preparations containing 5% (w/w) PG + cineole (C). PPL was contained in the films at 1 mg/cm^2. Cumulative PPL amount released was 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 (◊).

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