Feasibility of Transdermal Delivery of Prochlorperazine

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Prochlorperazine (PCPZ) is used as a drug of first choice to treat opioid-induced nausea and vomiting. To examine the feasibility of the development of a transdermal drug delivery system for PCPZ, we performed an in vitro skin permeation study with hairless mouse skin. When the concentration of 1-menthol in the hydrogel was 0.05%, the PCPZ flux was small; on the other hand, the flux was increased remarkably when the 1-menthol concentration was higher than 1%. The optimal formulation of hydrogel would be contained 20% isopropanol (IPA), 10% N-methyl-2-pyrrolidone (NMP), 2% 1-menthol and 1% PCPZ. The strong inhibitory effects to stereotyped behavior were observed at 4 h after administration of PCPZ hydrogel, and the efficacy was sustained for at least 8 h after the administration in mice in vivo. Thus, it was considered that PCPZ was delivered to brain via systemic circulation by the administration of PCPZ hydrogel.

Key words prochlorperazine; transdermal delivery; hairless mouse skin; hydrogel; 1-menthol

Nausea and vomiting are distressing symptoms associated with gastrointestinal disorders, pain following surgery, cancer chemotherapy, and the use of opioids. In the treatment of nausea and vomiting, dopamine receptor antagonists like prochlorperazine (PCPZ) and haloperidol are used.1-3 Particularly in palliative care, nausea and vomiting induced by the use of opioids become serious problems as side effects during the treatment of pain. The three-step analgesic ladder recommended by the World Health Organization (WHO) has been widely used in cancer pain management. According to WHO guidelines, strong opioids such as morphine are essential for the treatment of moderate-to-severe pain arising from cancer. In many cases, the use of morphine elicits nausea and vomiting by stimulation of the D2 dopaminergic receptor subtype located in the chemoreceptor trigger zone on the surface of the brain.2,4 PCPZ has been used as a first-choice drug to treat opioid-induced nausea and vomiting. The slow absorption and low bioavailability of oral administration derived from first-pass metabolism make it difficult to administer PCPZ orally for acute therapy.3 However, injection of PCPZ is short acting; frequent administration is necessary, and this causes a great deal of suffering for patients. Moreover, unexpected side effects appear when the plasma concentration of PCPZ is suddenly increased. Thus, many strategies to improve the bioavailability of PCPZ have been tried, including buccal administration, thermal aerosol inhalation, and an oral disintegrating film.5-9 Otherwise, for patients with dysphagia or difficulty with oral administration, alternative modes of PCPZ administration should be offered to increase therapeutic efficiency and quality of life.

In the present study, we tried to develop a transdermal delivery system for PCPZ, suggesting a possible approach to overcome the drawbacks of systemic PCPZ therapy via the oral and intravenous routes. Because transdermal drug delivery has inherent advantages such as (a) avoidance of first-pass metabolism, (b) control of infusion rate, (c) maintenance of plasma level depending on sustained release, (d) reduction of the side effects, and (e) prevention of gastrointestinal tract disturbance, it might be easier for elderly people and children than conventional administration. We investigated the feasibility of a transdermal drug delivery system (TDDS) for PCPZ in in vitro and in vivo methods.

MATERIALS AND METHODS

Materials Prochlorperazine maleate (2-chloro-10-[3-(4-methylpiperazine-1-yl)propyl]-10H-phenothiazine dimaleate) was a gift from Nippon Zoki Pharmaceuticals, Japan. 1-Menthol ((1R,25,5R)-(−)-menthol), amitriptyline hydrochloride (3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-1-propylamine hydrochloride) was purchased from Tokyo Kasei Kogyo Co., Ltd., Japan. Hydroxyethyl cellulose (HEC) was purchased from Sumitomo Seika Chemicals, Japan. Hydroxypropyl cellulose (HPC, nominal MW 1000000 Da), chloroform, methanol, isopropanol (IPA, 2-propanol), N-methyl-2-pyrrolidone (NMP, 1-methyl-2-pyrrolidone, for peptide synthesis) were purchased from Wako Pure Chemical Industries, Ltd., Japan. All other chemicals were of reagent grade and were used without further purification.

Preparation of Hydrogels The composition of the hydrogels used in this study is given in Table 1. HEC and HPC were added to distilled water and left overnight at room temperature for hydration. The rest of the components (% w/w) in the formula were then added to the HEC and HPC and dispersed by agitating overnight slowly with stirring until the mixture was completely homogeneous.

In Vitro Skin Permeation Study

Permeation Study The permeation studies were conducted with excised intact hairless mouse skin (Hos/HR-1 male, seven-week-old mice, Japan SLC) as a diffusion membrane. The diffusion cell used in the study was a modified Franz cell. The receiver compartment had a volume of 16 ml, and the effective surface area was 2.01 cm². The receiver so-

<table>
<thead>
<tr>
<th>Material</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prochlorperazine (PCPZ)</td>
<td>1</td>
</tr>
<tr>
<td>Isopropanol (IPA)</td>
<td>20</td>
</tr>
<tr>
<td>1-Menthol</td>
<td>2</td>
</tr>
<tr>
<td>N-Methyl-2-pyrrolidone (NMP)</td>
<td>10</td>
</tr>
<tr>
<td>Hydroxyethyl cellulose (HEC)</td>
<td>1</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose (HPC)</td>
<td>1</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
</tr>
</tbody>
</table>

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lution was a pH 7.4 phosphate buffer solution containing 4% (w/w) Brij 98 to maintain sink condition throughout the permeation study. The receiver solution was stirred by a magnetic bar and was maintained at a temperature of 37±1°C using a thermostatically controlled circulating water bath. One gram of hydrogel was applied under occlusive conditions. A 200 μl aliquot of receiver solution was periodically withdrawn following by replacement of the same volume of fresh, preheated receiver medium. Samples (200 μl) were frozen at −10°C prior to assay of PCPZ using HPLC.

**Determination of PCPZ** The determination of PCPZ was performed using an HPLC system (Hitachi High-Technologies, Japan) with UV detection at 254 nm. Aliquots (50 μl) of the sample containing the PCPZ analyte were injected onto an Inertsil CN-3 reversed-phase column (250×4.6 mm, 5 μm particle size, GL Sciences, Japan) that was eluted in isocratic mode with a 0.005 m ammonium acetate buffer (pH 5.25) containing methanol (15:85 v/v). The flow rate remained at 1.6 ml/min throughout the assay.

**Preparation of PCPZ Sample Solution for HPLC Assay** PCPZ in sample solution was quantified using a previously published method with some modifications. An aliquot (100 μl) of sampled receiver solution was mixed with 20 μl of amitriptyline hydrochloride in methanol (used as the internal standard at a concentration of 0.25 μg/ml), and 20 μl of 1N sodium hydroxide was added to make the mixture alkaline. One milliliter of chloroform was added, and the mixture was vortexed for 1 min. Subsequently, the mixture was centrifuged. The organic layer was evaporated under a stream of nitrogen at 39°C. The residue was reconstituted in 100 μl of methanol, and 50 μl was injected onto the HPLC column. Calibration curves for PCPZ, ranging from 0.0004 to 40 μg/ml, were prepared by spiking blank receiver solution with known amounts of PCPZ and amitriptyline.

**In Vivo Percutaneous Absorption Study in Mice**

**Animals and Drugs** Seven-week-old male ddY mice weighing 32—35 g, purchased from Japan SLC, were used for the percutaneous absorption study. The mice were housed weighing 32—35 g, purchased from Japan SLC, were used for the percutaneous absorption study.

**Experimental Procedure** Under pentobarbital anesthesia by intraperitoneal injection (50 mg/kg; Dainippon Pharmaceutical Co., Ltd., Japan), on the day before the start of the experiment, the hydrogel administration areas on the backs of the mice were shaved without damaging the skin. The mice were anesthetized with pentobarbital, and a glass cell with an effective diffusion area of 2.01 cm² was attached to the hair-free dorsal region using an adhesive (Daichi Sankyo Co., Ltd., Japan). Subsequently, the cell was filled with hydrogel (1 g) and capped with a plastic lid and Parafilm (Pechiney Plastic Packaging Inc., Canada). At 4 h and 8 h after application of the hydrogel, apomorphine was administrated subcutaneously, and the mice were then placed individually in a corner of an open field. The open field was a plastic box (27 cm W×33 cm D×15 cm H) with an open top; the floor was divided into five sections. A circle was drawn in the center of the open field, and two lines were drawn diagonally, to make five sections. The mice were allowed to explore the environment freely for 5 min and were observed to record two different apomorphine-induced components of stereotyped behavior: rearing and quick running. The activity was evaluated as follows. Rearing was scored 1 when mice stood upright with two forepaws free from the floor or touched the side surface of the apparatus with two forepaws, and it was scored 0 when mice had four paws on the floor. Quick running was scored 1 when mice crossed from one section to another, and it was scored 0 when mice were immobile or crossed to another section incompletely. During the experiment, mice were housed in cages and provided water ad libitum. In this study, apomorphine administration was conducted without anesthetization.

**Data Analysis** The steady-state flux (mg/cm²/h) was determined from the slope of the linear portion of the plot of the cumulative amount of drug permeating the skin per unit surface area against time. All the data obtained in the in vitro and in vivo experiments and stereotyped behavior were compared using Student’s t-test.

**RESULTS AND DISCUSSION**

**Effect of Components in the Formulation on the in Vitro Skin Permeation of PCPZ** The barrier function of the skin is maintained by the intercellular lipids of stratum corneum and thus it is difficult for drugs to penetrate the skin. We have investigated the abilities of cyclic monoterpenes and related compounds together with ethanol to facilitate percutaneous absorption and skin permeation of drugs in in vitro and in vivo studies. Moreover, the change in the lipid arrangements of the stratum corneum by the administration of transdermal absorption enhancers was investigated using synchrotron X-ray diffraction. Those results suggested that the change in lipid structure in the stratum corneum closely related to enhancing activity of drugs through skin. In the previous study, it was suggested that the combination of 20% IPA, 10% NMP and 2% l-menthol was very effective in transdermal delivery of tramadol (unpublished data). According to those results, the hydrogel formulation was decided as shown in Table 1. Flux was chosen for the evaluation of skin permeability and was estimated from the slope of the permeation profiles deduced from Fick’s law of diffusion by Eq. 1:

$$J = \frac{dM}{S \cdot dt}$$ (1)

where J is steady-state flux (mg/cm²/h), S is the effective diffusion area (cm²), and dM/dt is averaged as the total mass transport over the time course of the experiment (mg/h). Firstly, the effect of concentration of l-menthol in the hydrogel was evaluated. Figure 1 shows the effect of the concentration of l-menthol on the flux of PCPZ. When the concentra-
tion of L-menthol in the hydrogel was 0—0.5%, the PCPZ flux was small; on the other hand, the flux was increased remarkably when the L-menthol concentration was higher than 1%. However, the PCPZ flux slightly decreased when 5% L-menthol was added to the hydrogel. From the appearance of the hydrogel, it was speculated that L-menthol was saturated at approximately 2%, and therefore, the thermodynamic activity of L-menthol was maximized at 2% in the hydrogel. When the concentration of L-menthol was increased to 5%, an oily L-menthol phase was formed and acted as a reservoir of PCPZ, which resulted in a concentration gradient of PCPZ over the skin. Effective enhancement by L-menthol has been reported using a combination of solvents which behave synthetically and concentration dependency.13) However, the lipophilicity of drugs, enhancer and solvent could be closely related to exhibit synergistic and concentration dependency.

The change in alcohol and effect of concentration of alcohol on the skin permeation of PCPZ was evaluated as shown in Fig. 2. Decrease in amount of alcohol in the formulation could be decrease in skin irritation caused by the components of the formulation. However, the flux of PCPZ was very small when 10% IPA was formulated in the hydrogel. Moreover, the change in IPA to ethanol, the flux was almost same. Thus, 2% L-menthol and 20% IPA was considered to be necessary to maintain the adequate skin permeation of PCPZ. Those results were thought to be related to thermodynamic activity of L-menthol in the hydrogel.10) However, the concentration of L-menthol in the skin was also taken into consideration, thus, the further investigation should be necessary. In general, the dermal irritancy of ethanol is greater than that of IPA,14) we selected IPA as the solvent in the hydrogel. We investigated the effect of NMP on the flux of PCPZ (data not shown). Otherwise the flux was slightly changed by the addition of 10% NMP, the deviation of flux was decreased in comparison to without NMP in the hydrogel. It was considered that NMP was effective to the homogeneity of hydrogel and decrease in irritation of skin caused by the components of hydrogel. As a next, the concentration of PCPZ in the hydrogel was evaluated. As shown in Fig. 3, the flux was remarkably increased above 0.75%. It was considered that the thermodynamic activity of PCPZ in the hydrogel became maximized above 0.75%. Acceding to those results, the optimal formulation of hydrogel might be contained 20% IPA, 10% NMP, 2% L-menthol and 1% PCPZ in this experimental condition.

The Inhibition of Apomorphine-Induced Stereotyped Behaviors by the Administration of PCPZ Hydrogel

An open field test is commonly used in the realm of behavioral pharmacology, and it is utilized in the pharmacological study of the centrally acting agents of various behavioral actions. The behaviors of standing up and sniffing can be seen frequently and slight motor inhibition is also observed in control mice in an open field. On the other hand, remarkable biased behavior is observed in behavioral disorders mice. In this study, we assessed PCPZ hydrogel efficacy by observation of apomorphine-induced behavioral disorders in mice. Apomorphine is a nonselective dopaminergic agonist acting at five receptor subtypes (D₁—D₅), but it has a considerably weaker affinity for D₁ and D₅ than D₂, D₃, and D₄.15) It is mainly the D₂ dopaminergic-receptor subtype that is stimulated by the administration of apomorphine, and as a result biased locomotor behaviors are induced in mice.17) PCPZ has an inhibitory effect on the apomorphine-induced stereotyped behaviors because of its peripheral D₂ dopaminergic-receptor subtype antagonist activity, the frequency of these behaviors is expected to decrease with the onset of the pharmacological effect of PCPZ.18) Figure 4 shows inhibitory effect of PCPZ on the apomorphine-induced stereotyped behavior at 4 h and 8 h after the application of PCPZ hydrogel. The strong in-
hibitory effects were observed at 4 h after administration of PCPZ hydrogel, and the efficacy was sustained for at least 8 h after the administration. Bisedes, administration of hydrogel without PCPZ had no effect. Thus, it was considered that PCPZ was delivered to brain via systemic circulation by the administration of PCPZ hydrogel. L-Menthol elevates the threshold of sensitivity to cool temperature by stimulating the transient receptor potential cation channel subfamily M membrane 8 (TRPM8) receptor-activating channel of temperature receptors in skin nerves. It has been reported that TRPM8 and its central mediators, as elements of endogenous-cooling-induced analgesia, represent a novel analgesic axis that could be exploited in chronic sensitized pain states. Thus, the inhibitory effects might be caused by the analgesic action of L-menthol. But very active stereotyped behaviors were observed in mice to which L-menthol-containing hydrogel without PCPZ (control) had been applied, even 8 h later. Therefore, L-menthol did not have the inhibitory effects against the apomorphine-induced stereotyped behaviors in mice in this experimental condition. 

CONCLUSION

The results obtained from in vitro studies using the PCPZ hydrogels showed that skin permeability increased significantly with 2% L-menthol, 20% IPA, 10% NMP and 1% PCPZ. In vivo study in mice using the optimal hydrogels revealed that apomorphine-induced stereotyped behaviors could be inhibited by PCPZ. From those results, the feasibility of transdermal delivery of PCPZ was confirmed.

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REFERENCES