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Chitosan Gel for Transdermal Delivery of Morphine Hydrochloride

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We developed a chitosan (CHI) gel containing alcohols and *l*-menthol (MEN) as skin permeation enhancers, and succinic acid (SUC) as a cross-linking agent and examined the permeation of morphine hydrochloride (MPH) from the CHI gel into human skin. The solubility of CHI and MEN was measured beforehand in order to create a system saturated with these 2 substances. Solubility varied with the content of ethanol and 1,3-butylene glycol. The saturated CHI gel displayed sufficient elasticity to be applied to a fixed area of skin. The permeation of MPH into the skin from the CHI gel was compared with its permeation into the skin for a polyvinyl alcohol (PVA) gel having a similar composition. Permeation was greater for the CHI gel in the later stages of the experiment. Our CHI gel should thus be useful for the transdermal delivery of MPH.

Key words —— chitosan, *l*-menthol, ethanol, skin permeation enhancer, morphine hydrochloride

Introduction

Effective pain control for cancer patients is very important and many different types of drugs are used according to the guidelines[1]. Narcotic analgesics play a major role in pain control and many different types of preparations of opium alkaloids and related compounds are used depending on the condition of the patients[2]. Fentanyl is a synthetic opioid related to the phenylpiperidines and a transdermal delivery system is useful to obtain a prolonged action[3—5]. Since transdermal fentanyl therapy usually follows oral morphine therapy under stable conditions and morphine is mainly used as a rescue treatment for sudden pain suffered by patients using transdermal fentanyl[6,7], a transdermal delivery system for morphine will be especially useful to improve the pharmaceutical management of cancer patients. However, morphine is known to have a low permeability through the skin because of its hydrophilic nature[8].

The skin permeation of morphine hydrochloride (MPH) was found to be enhanced to a level providing a therapeutic effect by the combined use of alcohols, *l*-menthol (MEN) and water[8, 9]. For the development of a transdermal delivery system of MPH involving a mixed solvent system, a method for the application of the system is needed to retain the drug and enhancers until application and then release them into a selected area of skin after application. Gelation of the system is one possible way of preparing a monolithic matrix for application to the skin. Chitosan (CHI) is useful for the gelation of mixed solvents consisting of alcohols and water, and the relationship between the composition of the solvents and the visco-elasticity of the gel has been investigated[10]. In addition, CHI enhances the permeation of MPH through excised rat skin by a Donnan equilibrium effect[11]. Since CHI has been used as a safe biomaterial in medical and cosmetic fields[12, 13] and is impermeable to skin because of the large molecular weight, CHI is a possible candidate material for the transdermal delivery of MPH.

In the present study, CHI gel containing MPH, alcohols, MEN and water was prepared and the *in vitro* permeation of MPH through human skin was examined. Succinic acid (SUC) was used as a crosslinking agent. The chemical structure of the gel is shown in **Fig. 1**.

Materials and Methods

1. Materials

CHI (Marine Dew PC-100) was supplied by Ajinomoto

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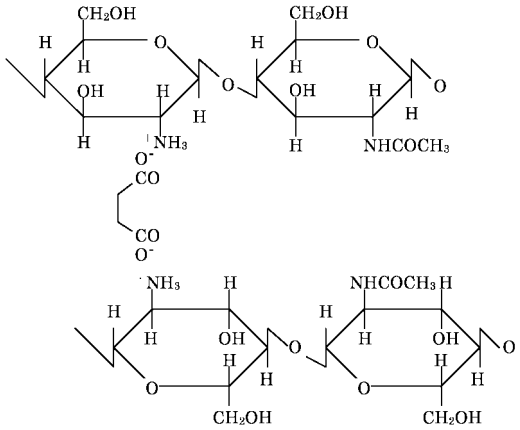

Fig. 1. Chemical Structure of the Gel Formation.

Co., Inc. (Tokyo, Japan) with a degree of deacetylation of about 50%. MPH was purchased from Takeda Chemical Industries, Ltd. (Osaka, Japan). MEN, ethanol (EtOH), SUC and hydroxypropylcellulose (HPC, HPC-M) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyvinyl alcohol (PVA, PVA-117) was purchased from Kuraray Co., Ltd. (Tokyo, Japan). 1,3-Butylene glycol (BG) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade.

2. Evaluation of the solubility of CHI in mixed systems

Various amounts of CHI were added to several mixed solvents consisting of EtOH, BG and water containing 2% SUC in a transparent glass vial. They were mixed well and then stored at 32°C. The transparency of the solution stored for 48 hr was checked by eye. The maximum concentration of CHI which retained the uniformity and transparency of the solution was regarded as the CHI solubility[10].

3. Evaluation of the solubility of MEN in CHI gels

Various amounts of MEN were added to several mixed solvents consisting of EtOH, BG and water containing 2% SUC and 1 or 3% CHI in a transparent glass vial. They were mixed well and then stored at 32°C. The phase separation of the solution stored for 48 hr was checked by eye. The maximum concentration of MEN which produced a clear solution was regarded as the MEN solubility in the gel.

4. Evaluation of CHI gelation

Stress relaxation of the CHI gel was measured by a rheometer (NRM-2002 J, Fudo Kogyo Co., Ltd., Tokyo) with a circular flat plunger (diameter 20 mm, thickness 1 mm) joined to a sensor and a rising sample stand[10]. The CHI gel (20 g) in a glass vial (volume 50 mL, internal diameter 32 mm, height 75 mm) rose at a rate of 30 mm/min and stopped at a strain in the range of 0.02 to 0.2. The elastic modulus of the CHI gel was calculated using the following equation :

$$E = \frac{F \cdot L}{a \cdot l}$$

where E and F are the elastic modulus and stress, respectively, L and l are the initial length and compressed length of the CHI gel, respectively, and a is area of the plunger. The time-courses of the F values were measured at four different strains, and the highest value immediately after compression and the constant value at $t=\infty$were noted for each measurement. The maximum value of E (E_0) and the E value at $t=\infty$(E_∞) at a strain of 0.04 were calculated from the corresponding F value obtained by a regression equation of the F values against the strains. E_0 and E_∞/E_0 were used as the indices of gel strength and maintenance of shape, respectively. For a completely elastic body, E_∞/E_0 is unity.

5. Preparation of transdermal delivery systems of MPH

A CHI gel disk containing MPH was prepared as described above. A silicone rubber mold was used for the preparation. The composition of the gel is shown in **Table 1**. In order to compare the CHI gel with gel consisting of non-ionic polymers, a PVA gel was prepared (Table 1). PVA was initially dissolved in boiled water to obtain a 20 % PVA solution. The cooled PVA solution was mixed well with other components and added to the mold. Three cycles of freezing (8 hours, −20°C)and thawing (8 hours, room temperature) were carried out to increase the elasticity of the gel[14].

6. Human skin permeation of MPH from CHI gel and PVA gel

Human skin obtained from HAB (Tokyo) was mounted on a Franz-type diffusion cell. It had 0.95 cm^2 of effective area and an open donor cell. Saline (4.5 mL) was added to the receptor compartment of the cell and this compartment was kept at 37°C. CHI gel or PVA gel was applied to the skin and the top of the gel was covered to avoid evaporation of the solvents. At predetermined intervals, samples were withdrawn from the receptor compartment for assay.

The concentration of MPH was assayed by an HPLC system (pump, LC-9A ; UV detector, SPD-6A ; Shimadzu Seisakusho, Kyoto, Japan). A LiChrospher 100 RP-18(e) 5 μm column (4.0×250 mm, Kanto Chemical Co., Tokyo, Japan) was used. A mixture of 0.1% phosphoric acid-acetonitrile

Table 1. Transdermal Delivery Systems of MPH Containing EtOH, BG, Water and MEN.

	CHI gel	PVA gel
Gel weight	300 mg	300 mg
Effective area	0.95 cm^2	0.95 cm^2
MPH	2 %	2 %
EtOH	20 %	20 %
BG	30 %	30 %
l-MEN	5 %	5 %
CHI	3 %	−
SUC	2.5 %	−
PVA	−	5.6 %
HPC	−	1.5 %
Water	37.5 %	35.9 %

(65 : 35) containing 5 mM sodium dodecylsulfate was used as the mobile phase, and elution was performed at 40°C with a flow rate of 1.2 mL/min. Aliquots of 200 μL of the receiver solution were mixed with the same volume of methanol containing naloxone hydrochloride as an internal standard, and then the mixture was centrifuged at 5000 g for 10 min. Aliquots of the supernatant (20 μL) were injected into the HPLC system. The UV detector was operated at 230 nm.

Results

1. Solubility of CHI in mixed systems

The solubility of CHI was examined in mixed systems containing various concentrations of EtOH and BG. The solubility values are shown in **Fig. 2**. The solubility of CHI in water containing 2% SUC was about 16% and the highest in this series. Addition of both EtOH and BG reduced the solubility of CHI because of its hydrophilicity. The effect of EtOH was greater than that of BG, since EtOH is more lipophilic than BG.

2. Solubility of MEN in CHI gel

The thermodynamic activity of MEN in vehicles is important for efficacy as a skin permeation enhancer[15]. The solubility of MEN in the CHI gel was measured to determine the saturated concentration in the mixed solvent systems. The seven different compositions in Fig. 2 were chosen for the determinations. The solubility of CHI in four of them was about 3%, that in a further two was about 4% and that in the remaining one was about 5%. The concentration of CHI in the examined gel was 1 or 3%. The solubility of MEN in the gel varied (0.5–6.4%) depending on the content of EtOH and BG (**Fig. 3**) while the concentration of CHI appeared to have no effect. Since MEN is lipophilic, a higher solubility of MEN was observed in compositions in which the solubility of CHI was lower.

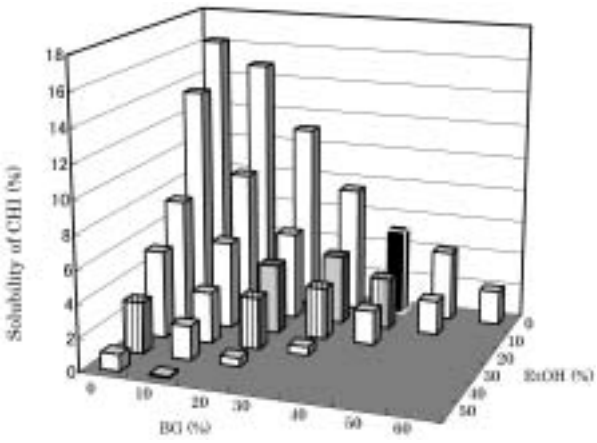

Fig. 2. Solubility of CHI in Mixed Systems Containing EtOH, BG and 2% SUC at 32°C.
The compositions (stripe, gray and black) are shown in Figs. 3 and 4.

3. Elastic properties of CHI gel

The elastic properties of seven kinds of CHI gel with different compositions were examined using a rheometer. The MEN content in each gel was almost equal to the solubility of MEN in the corresponding solution (Fig. 3). The CHI content of the gel was fixed at 3%. The values of E_0 and E_∞/E_0 of the gel with 20% EtOH, 30% BG and 5% MEN, 30 % EtOH, 20% BG and 3% MEN, and 40% EtOH and 5 % MEN were relatively higher than the others (**Fig. 4**). They were suitable for application to a fixed area of skin.

4. Skin permeation of MPH from CHI gel

CHI gel containing MPH was developed based on the results described above. PVA gel was also prepared to allow a comparison with the CHI gel. The MPH permeation from CHI gel and PVA gel through human skin is shown in **Fig. 5**. The permeation of MPH during the early phase was similar in both gels, but that during the later phase was different. The permeation rate of MPH from CHI gel was more constant than that from PVA gel (Fig. 5 B). This sustained permeation of MPH from CHI gel could be helpful for continuous pain control.

Discussion

Since CHI is a hydrophilic polymer, the solubility in water could fall with the addition of organic solvents[10]. From our results, the solubility in the mixed systems consisting of water, 2% SUC, EtOH and BG varied depending on the content of EtOH and BG (Fig. 2). A total concentration of EtOH and BG of 40–50% in a vehicle could be suitable for the transdermal delivery of MPH[16]. The solubility of CHI in the systems with such concentrations of EtOH and BG was about 3–4%. A CHI concentration of 3–4% appeared to be suitable for a gel preparation for skin application.

MEN acts as a skin permeation enhancer, when it is used with alcohols[17–19]. In this action, the potential of the enhancing effect depends on the thermodynamic activity of MEN in the systems[20]. A saturated solution of MEN would be needed to obtain a higher enhancing effect. In this study, the solubility of MEN in CHI gel with different compositions was measured. The solubility of MEN in the gel varied (0.5–6.4%) depending on the content of EtOH and BG, but was unaffected by the CHI content (Fig. 3).

The elastic properties of the CHI gel consisting of alcoholic solutions with saturated MEN were measured (Fig. 4). In our previous report, the elasticity of the CHI gel depended on the ratio of the actual concentration to the solubility of CHI in the systems[10]. A similar trend was observed in Fig. 4. The CHI gel with 3% CHI and 2% SUC containing 20% EtOH, 30% BG and 5% MEN, 30% EtOH, 20% BG and 3% MEN, or 40% EtOH and 5% MEN showed sufficient elasticity for application to skin.

A CHI gel containing MPH was prepared and the human skin permeation of MPH from the CHI gel was compared

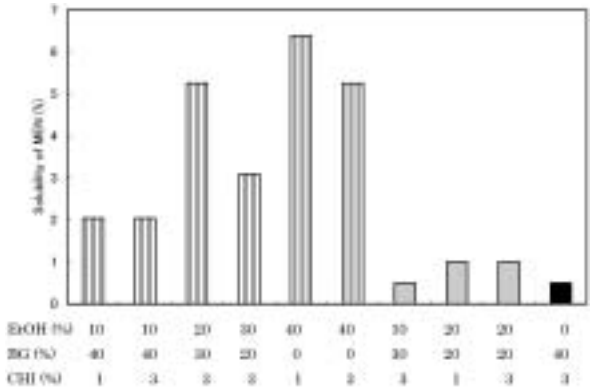

Fig. 3. Solubility of MEN in Mixed Systems Containing EtOH, BG, CHI and 2% SUC at 32°C.
Solubility of CHI in the systems (stripe, gray and black) were about 3, 4 and 5%, respectively (Fig. 2).

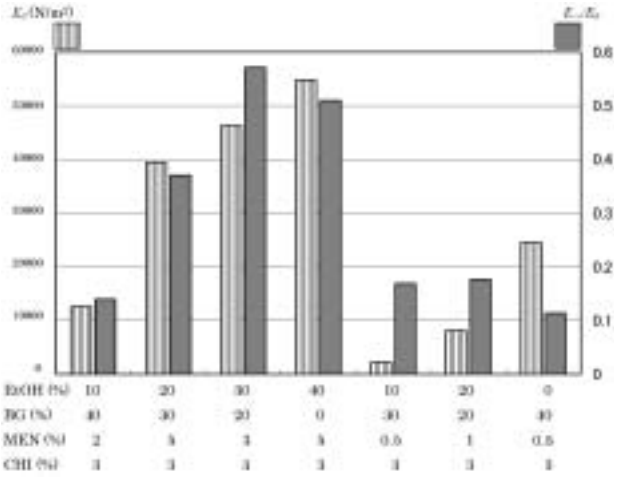

Fig. 4. E_0 and E_∞/E_0 of 3% CHI Gel Containing EtOH, BG, MEN and 2% SUC.
Stripe, E_0; gray, E_∞/E_0.

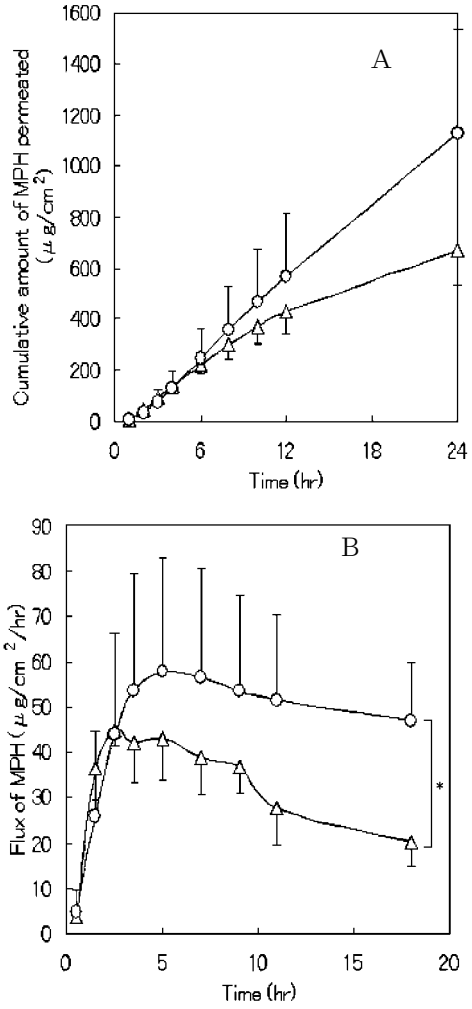

Fig. 5. *In Vitro* Permeation of MPH from CHI Gel or PVA Gel through Human Skin.
A, cumulative amount of MPH; B, flux of MPH.
○, CHI gel; △, PVA gel. Each data point is the mean+SE (n=3). *$p<0.05$.

with that from PVA gel having a similar composition. In our previous study, cationic polymers involving CHI had an enhancing effect on the permeation of MPH from mixed solvent systems containing MEN and EtOH through excised rat skin[11]. This phenomenon could be related to the Donnan effect of charged polymers[21]. In this study, the permeation of MPH from CHI gel during the later phase was higher than that from PVA gel without charged polymers. The sustained permeation of MPH from CHI gel could be helpful in pain control and the higher permeation during the later phase could also be related to the Donnan effect. The release of positively charged morphine is increased in the vehicle containing the positively charged CHI based on the Donnan equilibrium theory, and then the distribution of morphine into skin increases to enhance the transdermal absorption. Such an enhancing effect should be safer than that of low molecular weight enhancers because CHI acts outside the body, while the low molecular weight agent is absorbable. When MPH in a hydroxypropylcellulose gel containing similar MEN-EtOH-BG mixed enhancer system was applied to the skin surface of patients with pain, the pain was controlled but slight erythema was observed in 3 out of 4 patients[22]. If a higher absorption of morphine were needed to increase the pharmacological effects, a stronger permeation-enhancing effect would be needed. However, additional use of MEN, EtOH and/or BG could induce more severe skin erythema. Therefore, CHI as an impermeable safe enhancer is a suitable material for use in a transdermal MPH delivery system.

In conclusion, a CHI gel containing MPH was developed in this study. The thermodynamic activities of CHI and MEN were sufficient to allow suitable elasticity of the gel and permeation of MPH through the skin, respectively. In order to optimize both conditions, several experiments were performed and, eventually, a CHI gel having optimum properties was obtained. This CHI gel will be useful for the transdermal MPH delivery.

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