Chitosan Hydrogel as a Base for Transdermal Delivery of Berberine and Its Evaluation in Rat Skin

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Berberine is the main ingredient of Coptis spp. This study selected berberine as a model drug to design a transdermal delivery system for the treatment of cutaneous leishmaniasis. Berberine was incorporated into chitosan hydrogel to prepare ointments. The physicochemical properties of the ointments and the release profile of berberine were investigated. The results indicated that the viscosity of chitosan hydrogel increased with an increasing amount of lactic acid or EDTA. The effect of EDTA on the viscosity was greater than that of lactic acid. By differential scanning calorimetry (DSC) measurement, no interaction was found to occur between chitosan and the soluble berberine. The release rate of berberine was inversely proportional to ointment viscosity. In in vitro skin perfusion studies, only trace amounts of berberine permeated through the rat skin due to its low oil-water partition coefficient. Surfactants were used as penetration enhancers to increase the percutaneous absorption of berberine. Among the enhancers, benzalkonium chloride was found to be the most efficient. Additionally, Tween 80 could increase the loading amount of berberine in the skin.

Key words: Coptis spp.; berberine; chitosan hydrogel; transdermal delivery; rat skin; in vitro

Berberine [1,8,13,13a-tetrahydro-9,10-demethoxy-2,3-(methylenedioxy)-beberinium] is an alkaloid which is the main ingredient of Coptis spp. It has multiple pharmacological effects in anti-platelet coagulation, anti-inflammation and anti-tumor. A wide spectrum of anti-bacterial effects to G (−) bacillus, E-coli, dysentery bacillus, salmonella and staphylococcus has also been reported. An in vitro study showed that both Coptis spp. and berberine play the same roles in bacterial inhibition under low concentration and bactericidal effect under high concentration. Recently, Ghosh et al. found that berberine had a strong effect in inhibiting leishmania activity. More than twelve million infected cases and five thousand victims of leishmaniasis disease have been reported in Asia, Africa and Latin America in recent years. Over 400000 new cases are reported every year, but the number of available chemotherapeutic drugs is small because of their severe side effects. However, berberine and its derivatives are good candidates for treatment of leishmaniasis disease.

With respect to the pathogenesis of leishmaniasis, the organisms disseminate widely and cause nodules as well as plaque-like lesion under the skin several months after the initial infection by sandfly. The application of transdermal delivery systems to cure cutaneous leishmaniasis, besides the ease of self-administration and increased efficiency, may increase the therapeutic index of the drug. To develop a transdermal delivery system for berberine, vehicle effects may have a profound influence upon the topical products. It is well known that chitin (1,4 linked 2-acetamide-2-deoxy-β-d-glucagan) is abundant in nature. Chitosan is a polycationic polymer and an N-deacetylated product of chitin. This natural polysaccharide possesses useful properties such as non-toxicity, high biocompatibility and non-antigenicity that offer advantages for possible clinical use. More recently, it has also been shown that chitosan is a mucoadhesive and enhances the penetration of macromolecules across the intestinal and nasal barriers. Besides, chitosan has shown inductive and stimulatory activity on connective tissue-rebuild-

Materials and Methods

Materials Berberine hydrochloride hydrate was purchased from Acros Chemical Co. (Pittsburgh, U.S.A.). Chitosan was purchased from Aldrich Chemical Co. (Steinheim, Germany). Lactic acid and polyoxyethylene sorbitan monoleate (Tween 80) was purchased from E. Merck Co. (Darmstadt, Germany). Sodium lauryl sulfate was purchased from Wako Chemical Co. (Osaka, Japan). Ethylenediaminetetraacetic acid disodium salt (EDTA), benzalkonium chloride hydrate and p-hydroxy-biphenyl were purchased from Sigma Chemical Chemical Co. (St. Louis, U.S.A.). All other chemicals were of analytical grade.

Skin Preparation Rat skins were harvested from male Wistar rats weighing 230—270 g. After being sacrificed in a CO2 chamber, the abdominal hair was removed with electric clippers. A 3×3 cm2 section of denuded skin with a thickness of 0.65—0.75 mm was excised immediately before the permeation experiment.

Preparation of Berberine Ointments Berberine ointments were prepared according to the formula in Table 1; chitosan powder was suspended in double distilled water and lactic acid was added while stirring the sample. The other samples were prepared as above by adding 0.3 g EDTA/or 0.2 g absorption enhancers respectively. Berberine was added to ointments to give a concentration of 0.1%.

Determination of Viscosity Viscosity studies were done...
Table 1. Formulae of Chitosan Ointments

<table>
<thead>
<tr>
<th>Composition</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine (g)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>Chitosan (g)</td>
<td>3.0</td>
<td>3.0</td>
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<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
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<tr>
<td>Lactic acid (g)</td>
<td>4.1</td>
<td>5.0</td>
<td>5.0</td>
<td>3.5</td>
<td>4.1</td>
<td>4.1</td>
<td>5.0</td>
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<tr>
<td>Na₂ EDTA (g)</td>
<td>---</td>
<td>---</td>
<td>1.5</td>
<td>1.5</td>
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<td>1.5</td>
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<tr>
<td>Benzalkonium chloride (g)</td>
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<td>---</td>
<td>---</td>
<td>1.0</td>
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</tr>
<tr>
<td>Sodium lauryl sulfate (g)</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>1.0</td>
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</tr>
<tr>
<td>Tween 80 (g)</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1.0</td>
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<tr>
<td>Add water to (g)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tr>
</tbody>
</table>

in the 24 h after ointments preparation. By using a Cone and Plate Viscometer (Brookfield Digital Viscometer, Model DVII), 0.5 g of ointment was placed in the sample cup of the viscometer and allowed to stand for 1 h to reach 37 °C. To obtain stable display readings, viscosity measurements were made 30 s later.

**Determination of pH Value**  The pH values of the chitosan hydrogel were determined the day after ointment preparation, by using pH Meter CG840 (Schott Co.).

**Differential Scanning Calorimetry (DSC)**  DSC thermograms were obtained by using a Perkin-Elmer DSC7 differential scanning calorimeter. Sample sizes were in the range of 1.5—3 mg and were sealed in a volatile type aluminum pan. Thermograms were recorded from 30 to 100 °C at a scan rate 5 °C/min.

**In Vitro Release Experiment**  Vertical-type diffusion cells were similar to the apparatus of the Franz diffusion assembly. One side of the cell was filled with berberine chitosan hydrogels; this side (donor cell) was separated with the Visking seamless cellulose tubing C-110 membrane from the other side (receptor cell), which was filled with phosphate buffer (pH 7.4, containing 20% w/w PEG 400). The area available for diffusion was 2.43 cm². The receiver compartment was agitated by a magnetic stirrer at 700 rpm. The apparatus was maintained at 37±0.5 °C with a water jacket. An aliquot (0.5 ml) of the sample was taken from the receiver compartment at appropriate times, and the concentration of berberine was determined by the spectrophotometer (Hitachi 2000 Hitachi Seisakusho Co., Ltd.) at 260 nm. After each sampling, the same volume of fresh phosphate buffer was added to the receiver compartment to keep the volume constant. The release of berberine from ointment was recorded continuously for 24 h.

**Determination of Permeation through Rat Skin**  The diffusion cells were used in a way similar to the apparatus of the release experiment. The excised rat skin was used as the membrane for skin permeation experiments. The skin was positioned with the stratum corneum facing the donor cell, and the dermis side was in contact with the receiver compartment. Samples (0.5 ml) were withdrawn from the receiver cell at appropriate time and the concentration of berberine was determined by HPLC.

**Measurement of Berberine Concentration in Rat Skin**  The amount of berberine remaining in the rat skin after the removal of ointments was determined after careful removal of the skin from the diffusion cells and rapid washing several times with distilled water. Then, the skin was weighed and homogenized in 3 ml lactic acid solution (pH 3.0) by means of a tissue homogenizer. The homogenate was centrifuged and the supernatant was filtered, then analyzed by HPLC.

**Analytical Methods**  The amount of berberine in each sample was determined by HPLC. p-Hydroxy-biphenyl was used as an internal standard. The conditions were as follows: pump, model L-6000 (Hitachi); column, 4.6×250 mm Spheris C18 (Phase Separations Ltd., U.K.); mobile phase pH 5.2 phosphate and citric acid buffer solution, acetonitrile (1:1 v/v); UV detector, model L-4000 (Hitachi); wavelength, 260 nm; flow rate, 1.2 ml/min. Peak areas were calculated by using a chromatographic integrator model D-2500 (Hitachi).

**Measurement of Apparent Partition Coefficient**  The degree of berberine partitioning between 1-octanol (oil phase) and pH 4.0 lactic acid aqueous solution (water phase) was determined as follows: 4 ml of berberine aqueous solution (0.10 wt % berberine in pH 4.0 lactic acid solution) and 4 ml of 1-octanol were placed in a glass-stoppered test tube and shaken in a water bath at 37 °C for 2 d. The mixture was centrifuged at 3000 rpm for 10 min. The berberine concentration in water was determined by the spectrophotometer at 260 nm.

**RESULTS AND DISCUSSION**

Chitosan can be dissolved in organic acids and the biologically safe lactic acid appeared to be a universal solvent for chitosan. The effect of EDTA and lactic acid amount on the viscosity of the ointment base is shown in Fig. 1. The results indicated that the viscosity increased with an increasing amount of lactic acid except for the EDTA addition, while the pH value decreased. This was probably caused by the lactic acid converting more glucosamine units of chitosan to soluble forms thus increasing its viscosity. As a complexing agent EDTA was added to the ointment base, and it was covalently bound to the primary amino groups of chitosan, and that caused the viscosity to increase. However, the effect of EDTA on the viscosity was greater than that of lactic acid. In addition, there was no significant difference in the viscosity of chitosan ointment after the introduction of soluble berberine. The DSC thermogram of ointments, normalized and adjusted to the same baseline, is given in Fig. 2. There is only one endothermic peak at around 55 °C. The thermogram obtained according to the chitosan hydrogel itself was the same as the one from the base containing berberine. DSC measurement showed that there was no interaction between chitosan and the incorporated drug.
The release profiles are shown in Fig. 3. The linear regression analysis with various models for each trial is summarized in Table 2. It was found that the best fit was obtained with the Higuchi equation, which gave for almost all the release curves the highest regression coefficient ($r^2$) and the smallest sum of weighed squared deviation. The release rate (flux) of berberine was inversely proportional to ointment viscosity. In other words, the increasing viscosity made it difficult for the berberine to diffuse through the gel-like base and into the external aqueous phase. Another important variable affecting the drug release was the amount of berberine incorporated with the other parameters being constant. By plotting the calculated fluxes against the percentage of berberine in the ointment, it was found that the drug release was proportional to the amount of berberine incorporated as shown in Fig. 4. The release kinetics of berberine from chitosan bases could be modeled by the Higuchi equation. Simultaneous DSC and drug release measurements indicated that neither chemical interaction nor crystallization occurred among the components in the ointments.

For formulation screening, the optimal product was observed when the hydrogel consistently possessed a clearer appearance, and was easier to apply to the skin. Thus, the range of viscosity was from 1.00 to 3.00 ($\times 1000$ cps). The pH value was over 3.0 to avoid skin irritation. In addition, there was a higher drug release rate among the chitosan hydrogels. Based on the above considerations, formulae No. 3 was chosen for further study to investigate the transdermal delivery of berberine in rat skin. In the in vitro skin perfusion
studies, the chitosan hydrogels containing berberine were applied on rat skin for 24h. It is recognized that the stratum corneum has the major diffusional barrier function for most compounds. Solute fluxes are assumed to be directly proportional to stratum corneum/water partition coefficients and diffusivities. However, the calculated apparent oil/water partition coefficient of berberine is 0.0103. Besides, the pH values of chitosan hydrogel applied are between 3.0 and 4.5. Stratum corneum did not undergo irreversible structural changes when treated with aqueous media at pH >3. Thus, only a trace amount of berberine had permeated through the rat skin as shown in Fig. 5. Corresponding to the release studies on these results, the transdermal permeation rate is much lower than the release rate of berberine from the chitosan-based hydrogels. Briefly, the permeation of berberine through the skin is the rate-determining step for percutaneous absorption of berberine from chitosan ointments. For this reason, penetration enhancers such as benzalkonium chloride, sodium lauryl sulfate and tween 80 were employed to increase the percutaneous absorption of berberine. In our previous report, it was shown that an anionic surfactant, sodium lauryl sulfate, had effective enhancement for some drugs to penetrate rabbit skin. However, the coagulas formed in chitosan hydrogel which became turbid after introduction of the oppositely charged sodium lauryl sulfate. The resulting product is unsuitable for study. The other penetration enhancers did not cause significant changes in the physicochemical properties of chitosan gel. Incorporation of either benzalkonium chloride or tween 80 into chitosan gels dramatically enhanced the percutaneous absorption of berberine (Fig. 5). The in vitro penetration parameters were calculated from the penetration data according to the methods of our previous report. Detailed information on percutaneous absorption enhancement of enhancers is given in Table 3. It has been generally found that cationic surfactants are more damaging to biological membrane than nonionic surfactants. Benzalkonium chloride which is a cationic surfactant showed the most pronounced effect on the penetration of berberine, yielding about a 6-fold increase in comparison with the control one without enhancer. Although the enhancement effect of benzalkonium chloride on berberine skin penetration is greater than that of tween 80, the opposite result was found in the cumulated amount of berberine in rat skin. This result is accordance with other reports, nonionic surfactants affecting the permeability of drug may be mainly attributed to a change of the vehicle/skin partition coefficient neither extraction of skin lipids nor denaturation of skin proteins.

In conclusion, the possible use of chitosan hydrogel as a new vehicle for transdermal preparation of berberine has been examined. The above results have demonstrated that berberine could penetrate into and through the rat skin in significant amounts after coadministration with absorption enhancers. Since berberine and Coptis spp. play similar roles in their pharmacological effects, further studies will employ the aqueous solvent extracts of Coptis spp. as a model drug to find out the effect of other constituents in Coptis spp. on transdermal delivery of berberine.

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