Preparation and characterization of polymeric micelles for topical delivery of itraconazole

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ABSTRACT SUMMARY
The capability of a series of amphiphilic copolymers (PEO-PCL-PEO) as a carrier for enhancing skin absorption of itraconazole (ITZ) was evaluated, using Poloxamer 188 as a control. Micelle formulations were evaluated by their size, encapsulation efficiency (EE), loading content (LC), release behavior, and skin permeation/deposition profiles. In vitro release rate and amount of ITZ from PEO-PCL-PEO micelles decreased as the CL/EO ratio increased. The in vitro skin permeation and deposition of ITZ decreased with the increase in CL/EO ratio. However, polymeric micelles (F1 and F2) showed significantly higher skin permeation and deposition of ITZ, compared to Poloxamer 188. These results demonstrate that polymeric micelles composed of amphiphilic PEO-PCL-PEO copolymers could be a useful carrier for topical delivery of ITZ, and can be optimized by the hydrophobic/hydrophilic block ratio.

INTRODUCTION
Itraconazole (ITZ) is a potent antifungal agent that has been widely used in clinics for various fungal infections. However, due to its low aqueous solubility, it is difficult to formulate a topical skin delivery system of ITZ that can maintain effective local tissue concentration. The objective of this study is to synthesize and evaluate PEG-PCL-PEG copolymer as a drug carrier in the form of polymeric micelles for enhancing percutaneous absorption of ITZ. The relationship between the structure of PCL and the property of polymeric micelles, including the particle size, encapsulation efficiency, release behavior and skin absorption, was systematically investigated. For comparison, commercial Pluronic® copolymer (i.e., PEG-PPG-PEG, Poloxamer 188) was studied under the same condition as the control.

EXPERIMENTAL METHODS
PEO-PCL-PEO triblock copolymers were synthesized following the literatures. Four different CL/EO ratios were used in synthesis to vary the hydrophobicity of copolymers. ITZ was then introduced into these copolymer micellar solutions using a tip-type ultrasonic processor. Firstly, ITZ and triblock copolymers were dissolved in methylene chloride (oil phase). Two types of continuous phase (water phase) were prepared in order to observe the effect of surfactant (i.e., Tween-80) on micellar properties; i.e., 10 mL of water only and 10 mL of water with 50 mg of Tween-80 (0.5 w/v %). The oil phase was added drop-wise to water phase, vigorously stirred at 500 rpm, and ultrasonicated for 2 min. Subsequently, methylene chloride was removed in a rotary evaporator at 60°C under a reduced pressure. Then, the micellar solutions were filtered through a Sartorius membrane (0.22 µm) to remove the unloaded ITZ.

Loading content (LC) and encapsulation efficiency (EE) of ITZ in micelles were determined by HPLC. Stability of micelles was observed by determining the change of the average particle sizes, where aqueous solution of ITZ-loaded polymeric micelles with Tween-80 was maintained under ambient temperature and mild agitation in the dark for 12 h using a shaking incubator.

The in vitro drug release study was conducted by placing a dialysis bag containing the drug-loaded micelle solution into phosphate buffer solution at 37°C. The in vitro skin permeation study across hairless mouse skin was conducted with Keshary-Chien diffusion cells at 37°C. The donor cells, contained
micellar solutions (1.0 mL) loaded with ITZ, were occluded with parafilm, and the receptor cells were filled with PBS and PEG400 (6:4, v/v) to maintain sink condition for ITZ. At the end of 12 h of the in vitro skin permeation experiment, the amount of ITZ retained in the skin was also determined.

RESULTS AND DISCUSSION

The average particle size of synthesized PEO-PCL-PEO series was smaller than those of Poloxamer 188, regardless of the presence of ITZ and Tween-80 (Table 1), probably due to the stronger hydrophobicity of PCL than PPO. In the case of synthesized PEO-PCL-PEO series, CL/EO ratio seems to govern the average particle sizes of micelles, incorporated drug percent and drug weight percent in micelles. This indicates that as the hydrophobic block ratio increased, the hydrophobicity of the micelles increased, resulting in higher ITZ amount in the micelle core. Moreover, it was interesting to note that Tween-80 significantly increased the drug loading in the triblock copolymer micelles. Tween-80 in ITZ-loaded polymeric micelles seems to increase the aggregation number and core volume, resulting in higher ITZ amount in the micelle core.

Table 1. The average particle sizes (nm) of polymeric micelles before and after incorporation of itraconazole with or without Tween-80. F5 is Poloxamer 188 as the control.

<table>
<thead>
<tr>
<th>Name</th>
<th>CL/EO</th>
<th>Drug(+) Tween-80(+)</th>
<th>Drug(+) Tween-80(+)</th>
<th>Drug(+) Tween-80(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:11</td>
<td>45±3.8</td>
<td>92±3.9</td>
<td>145.7±8.8</td>
</tr>
<tr>
<td>F2</td>
<td>0.200</td>
<td>53±6.8</td>
<td>129±13.8</td>
<td>188.7±16.7</td>
</tr>
<tr>
<td>F3</td>
<td>0.233</td>
<td>62±6.8</td>
<td>174±16.7</td>
<td>240.9±12.2</td>
</tr>
<tr>
<td>F4</td>
<td>0.331</td>
<td>72±6.6</td>
<td>191.9±14.9</td>
<td>314.2±25.5</td>
</tr>
<tr>
<td>F5</td>
<td>0.185</td>
<td>260.8±17.0</td>
<td>250.7±26.5</td>
<td>378.1±15.4</td>
</tr>
</tbody>
</table>

*Degree of polymerization: rounded off value determined by NMR.

The in vitro release rate and the amount of ITZ from PEO-PCL-PEO micelles linearly increased as the CL/EO ratio decreased (Figure 1). Because these micelles were fairly stable under ambient temperature for 12 h, weaker interaction between ITZ and hydrophobic PCL core of the micelles closely contribute to these results. Moreover, polymeric micelles (F1 and F2) significantly increased skin permeation and deposition of ITZ, compared to Poloxamer 188 (F5) (Figure 2).

CONCLUSION

In this study, a series of amphiphilic PEO-PCL-PEO triblock copolymers were prepared. The lower CMC and smaller particle size of PEO-PCL-PEO than Poloxamer 188 indicated that they develop stable micellar forms. PEO-PCL-PEO micelles (especially F1 and F2) showed higher release rate and amount, together with higher skin permeation and skin deposition of ITZ. Thus, polymeric micelles can be a useful skin absorption carrier for poorly water-soluble ITZ and can be optimized by the properties of polymers, including hydrophobic/hydrophilic block ratio.

Figure 1. In vitro release profiles of itraconazole from the polymeric micelles of synthesized PEO-PCL-PEO series (F1 to F4) and Poloxamer 188 (F5) containing 0.5% (w/v) Tween-80 (mean±S.D., n=3).

Figure 2. Amount of itraconazole in the skin at the end of 12 h of in vitro permeation studies (mean±S.D., n=3).

REFERENCES