Pharmacokinetics of a New, Orally Available Ceftriaxone Formulation in Physical Complexation with a Cationic Analogue of Bile Acid in Rats

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Oral administration of ceftriaxone associated with a bile acid-based new oral carrier, cholesterylendiamine, in 50% propylene glycol to rats at doses of 25 and 50 mg/kg of body weight resulted in a significant increase in intestinal absorption, as evidenced by 55% improvement in the bioavailability, whereas ceftriaxone alone showed a bioavailability of less than 1%.

The introduction of new oral cephalosporins, or formulation development of existing and highly promising parenteral cephalosporins, provides a possibility for follow-up oral treatment after initial parenteral treatment, thus reducing hospitalization time and costs (5, 6, 14). Ceftriaxone (CRO) is a widely used injectable broad-spectrum cephalosporin that exhibits potent activity against gram-positive and gram-negative bacteria (1, 2, 11, 12). However, the therapeutic utility of ceftriaxone is limited, as it requires parenteral infusion for its administration. In this respect, the development of an oral formulation of CRO would be helpful in rapid and proper step-down therapy that would lead to better patient compliance without compromising the therapeutic activity.

In a previous study, we developed a new oral delivery carrier based on bile acids, which could improve the oral bioavailability of therapeutically active peptides without damaging the tissue structure of the mucous membrane (8, 9). Based on the observation that associated bile acid could increase the absorption of a poorly absorbable drug in the intestine, we prepared a positively charged carrier by simple modification of bile acid, cholic acid, with ethylenediamine for the negatively charged small organic molecules, CRO. This scheme was designed with the goal of preparing a carrier that could physically associate with the drug by ion pair interaction to improve its lipophilicity without altering the structure of the native drug. In the present study, we show that the positively charged bile acid analogue forms a complex with CRO and report the impact of this association on the oral bioavailability.

Cholesterylendiamine (CEA) was synthesized by using cholic acid and ethylenediamine as precursors. A delivery agent was composed of the hydrophobic part of bile acid and the positive charge of ethylenediamine, thereby facilitating an ion-pairing interaction with anionic drug, CRO. The complex formation in aqueous medium was reversible and dependent on the molar ratio between CRO and the delivery agent. Since CRO/CEA complexes showed significantly reduced solubility in water, the lyophilized drug complex was reformulated by the addition of one of the most common water-soluble solvents, propylene glycol (PG) (15), as a prototype formulation.

The partition coefficients of CRO/CEA complexes were investigated in an n-octanol/water system. CRO concentrations were analyzed on a reversed-phase high-performance liquid chromatograph (RP-HPLC) (Shimadzu, Tokyo, Japan) as previously described (3). Briefly, a 50-µl aliquot of supernatant was injected into an RP-HPLC fitted with an Eclipse XDB analytical column (25-cm by 4.5-cm inside diameter; Agilent, Palo Alto, CA). The mobile phase was a mixture (50:50 [vol/vol]) of phosphate buffer (10 mM; pH 7) and acetonitrile (containing 0.64% tetraethyl ammonium bromide). The flow rate was 1 ml/min, and UV detection was performed at 272 nm. The method showed good reproducibility, with a coefficient of variation of less than 10%. As expected, CRO, a hydrophilic drug containing many polar groups, exhibited a very small log P value of −2.1 ± 0.19. However, the combination with CEA led to a significant improvement in the log P value (complex mole ratios of CRO and CEA at 1:1, 1:2, 1:3, and 1:5 increased the log P to −0.6 ± 0.03, −0.2 ± 0.01, −0.13 ± 0.05, and −0.21 ± 0.01, respectively). It was observed that the log P values did not change significantly beyond the molar ratio of 1:2; hence, we selected a CRO/CEA ratio of 1:2 for further studies.

The pharmacokinetics of CRO/CEA oral formulations were evaluated after intravenous (i.v.) and oral (p.o.) administration to rats that had fasted overnight. We followed the National Institutes of Health guidelines for the care and use of laboratory animals (11a). To extract CRO from plasma, 200 µl of plasma was spiked by the addition of 200 µl of distilled water and 800 µl of acetonitrile before injection into the RP-HPLC. The area under the curve (AUC) was calculated using the linear trapezoidal method. The plasma concentration-time curves of CRO and CRO/CEA complexes, administered intravenously to rats at 25 mg/kg of body weight per dose, are shown in Fig. 1A and are listed in Table 1. Consequently, the intravenously administered CRO/CEA complex formulation was able to return to the native drug form in the blood and showed approximately twofold-improved plasma exposure profiles (P < 0.01) compared to CRO alone. Furthermore, the clearance of the CRO/CEA complex was decreased about 50%; on the other hand, its half-life was increased twofold. One of the


reasons for these results might be the slow dissociation of CRO from its complexed CRO/CEA form.

After oral administration of aqueous CRO alone (50 mg/kg), no appreciable plasma concentration of CRO was detected. On the other hand, when the complex of CRO/CEA in PG was coadministered, an increase in the plasma CRO concentration was observed. Figure 1B shows the effects of reformulation based on the contents of PG in CRO/CEA complexes. The dose of oral CRO (50 mg/kg) was fixed, and three different CRO formulations (PG contents, 10, 50, and 80%, respectively) were orally administered. The bioavailability (F) was calculated by using the area under the curve after oral administration (AUC<sub>p.o.</sub>) relative to the area under the curve after intravenous administration (AUC<sub>i.v.</sub>) with the following equation: $F = \frac{[AUC_{p.o.} \times \text{dose}_{p.o.}]}{[AUC_{i.v.} \times \text{dose}_{i.v.}]}$. Among the several formulations studied, we chose CRO/CEA in 50% PG as a prototype oral CRO formulation (AUC<sub>0-8 h</sub> [the AUC from 0 to 8 h] for CRO/CEA at 10, 50, and 80% was 8.9 ± 5.8, 118.2 ± 39.0, and 72.0 ± 13.1 µg · h/ml, respectively). Figure 1C shows the dose-response profiles of the orally administered CRO/CEA formulation. Three different doses of the CRO formulation (12.5, 25, and 50 mg/kg) were associated with CEA. The increased amount of CRO formulation induced a dose-dependent enhancement of the plasma CRO concentration. It has been observed that the oral formulation at CRO equivalent doses of 12.5, 25, and 50 mg/kg induced enhancements in the peak plasma CRO concentration and the AUC<sub>0-8 h</sub>. The plasma CRO concentration and the AUC value were increased proportionally to the administered dose, with the oral bioavailability ranging from 30 to 55% (Table 2). At the fixed dosing volume, the viscosity of the drug solution was increased with the increasing concentration of the CRO/CEA complex in a viscous PG formulation. The increased viscosity of the drug solution might have increased its residence time in the gastrointestinal tract. Therefore, the plasma CRO concentration was sustained longer and its bioavailability appeared to be higher when the increased dose was orally administered with the fixed dosing volume (Fig. 1C).

Cholic acid is an endogenous substance consisting of a facially amphiphilic steroid nucleus with a hydrophobic α-side.

### Table 1. Pharmacokinetic parameters of CRO and CRO/CEA complexes administered intravenously at a dose of 25 mg/kg to rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CRO (in saline)</th>
<th>CRO/CEA (in 30% PG)</th>
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<tbody>
<tr>
<td>$C_{\text{ss}}$ (µg/ml)</td>
<td>204.89 ± 48.6</td>
<td>166.28 ± 56.3</td>
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<tr>
<td>$T_{1/2}$ (h)</td>
<td>0.67 ± 0.35</td>
<td>1.41 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-8 h&lt;/sub&gt; (mg · h/ml)</td>
<td>106.16 ± 12.8</td>
<td>199.37 ± 19.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cl (ml/h)</td>
<td>59.56 ± 7.7</td>
<td>31.53 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>$V_{\text{ss}}$ (ml)</td>
<td>43.88 ± 0.76</td>
<td>59.40 ± 7.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are presented as means ± standard deviations (n = 5).

<sup>b</sup> P < 0.01 compared to aqueous CRO.

<sup>c</sup> $C_{\text{ss}}$, drug concentration in plasma at time zero; $T_{1/2}$, half-life; AUC<sub>0-8 h</sub>, area under the concentration-time curve from 0 h to infinity; Cl, clearance; $V_{\text{ss}}$, volume of distribution at steady state.

### Table 2. Pharmacokinetic parameters following oral administration of escalating doses of oral CRO formulation to rats<sup>a</sup>

<table>
<thead>
<tr>
<th>Oral formulation</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC&lt;sub&gt;0-8 h&lt;/sub&gt; (µg · h/ml)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRO without CEA</td>
<td></td>
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<tr>
<td>50 mg/kg in PBS</td>
<td>0.8 ± 0.3</td>
<td>0.5</td>
<td>0.9 ± 0.5</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>50 mg/kg in 50% PG</td>
<td>1.8 ± 0.3</td>
<td>0.5</td>
<td>4.6 ± 1.4</td>
<td>4.3 ± 1.3</td>
</tr>
<tr>
<td>CRO/CEA in 50% PG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5 mg/kg</td>
<td>3.5 ± 1.7</td>
<td>1.0 ± 0.3</td>
<td>7.7 ± 3.0</td>
<td>14.6 ± 5.7</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>9.9 ± 3.9</td>
<td>1.2 ± 0.4</td>
<td>31.8 ± 15.1</td>
<td>30.3 ± 14.4</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>22.9 ± 8.1</td>
<td>2.2 ± 1.0</td>
<td>118.0 ± 39.0</td>
<td>55.7 ± 18.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are presented as means ± standard deviations (n = 5). $C_{\text{max}}$, maximum concentration of drug in plasma; $T_{\text{max}}$, time to maximum concentration of drug in plasma; AUC<sub>0-8 h</sub>, area under the concentration-time curve from 0 h to 8 h; F, oral bioavailability.
and a hydrophilic β-side (10, 13). The natural physicochemical and enterohepatic circulation properties of the bile acids could be utilized for the preparation of new drugs (4, 7, 8, 16). Our concept was to design a delivery carrier based on bile acid molecules that could physically interact with oppositely charged hydrophilic, and thus poorly absorbable, drugs. Results from animal studies have indicated that CEA significantly affected the pharmacokinetic parameters for orally administered CRO in a dose-dependent fashion. The mechanism of improved CRO absorption by CEA is partly explained by the physicochemical structures of drug complexes. Once CEA makes an ion-pairing interaction with CRO based on opposite electrostatic charges, it increases the lipophilicity and stability of the native drug by combining a hydrophobic steroid nucleus of bile acid with CRO. Increased lipophilicity improves the absorption of the drug by the mucosal membrane, and increased stability would prolong the therapeutic activity of the drug in the gastrointestinal region (8). Furthermore, these synthetic delivery carriers would maintain the biological activities of the therapeutic drug at a reduced enhancer concentration, because the enhancer directly interacts with drugs by electrostatic interaction, unlike other prodrug forms or conventional penetration enhancers.

In conclusion, a cholic acid derivative was prepared with the aim of enhancing the oral bioavailability of CRO. Preliminary data indicated that our synthesized novel carrier improved the absorption of CRO without compromising its biological activity; thus, we anticipate that it could serve as an alternative to injectable CRO with enhanced patient compliance.

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REFERENCES