Clinical Pharmacokinetics of Oseltamivir and Its Active Metabolite Oseltamivir Carboxylate after Oral Administration in Horses

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ABSTRACT. The aim of this study was to investigate the pharmacokinetics of oseltamivir carboxylate (OC) in horses (n=6) after oral administration of its prodrug oseltamivir. The binding rate of OC to horse plasma proteins was negligible (<1%). Oral administration of oseltamivir of 2 mg/kg body weight of oseltamivir to horses provided a plasma concentration of OC (mean maximum concentration: 257.9 ng/ml) above the inhibitory concentrations against equine influenza A viruses determined in vitro. However, because OC is rapidly eliminated from horse plasma (mean elimination half-life: 2.5 hr), administration intervals should be less than 10 hr to retain a suitable concentration when using a single dose of 2 mg/kg oseltamivir.

KEY WORDS: equine, oseltamivir, pharmacokinetics.

Equine influenza A virus (EIV) is an important pathogen in horses that causes an acute severe respiratory disease. Clinical signs include pyrexia, depression, anorexia, cough and nasal discharge, and occasional occurrence of secondary bacterial infections. Rapid transmission of the infection between animals is a characteristic of this disease [6]. Oseltamivir (Tamiflu™, Chugai Pharmaceutical, Tokyo, Japan) was recently introduced into clinical practice as a therapeutic and prophylactic agent for human influenza in various countries [1]. Oseltamivir is an ethyl ester prodrug. Orally administered oseltamivir is converted into its active metabolite (oseltamivir carboxylate, OC) by hepatic esterases and is exclusively excreted into urine without further metabolism in humans and some experimental animals (Fig. 1) [3]. We first reported that OC inhibits the neuraminidase (NA) activities and plaque-forming of several EIV strains in vitro [9]. Subsequently, we reported the therapeutic and prophylactic efficacies of oral administration of oseltamivir in horses experimentally infected with EIV [10]. However, since the pharmacokinetics of OC in horses after oral administration of oseltamivir remain unknown, we were obliged to adopt human dosage regimens in our previous study [10] according to the human studies described by Whitley et al. [7] and Hayden et al. [2]. In this study, to design a rational dosage regimen for oseltamivir in horses, we investigated the pharmacokinetic profiles of OC in horses after oral administration of oseltamivir. In addition, we examined the safety of multiple high doses of oseltamivir in horses.

Six healthy light-breed horses were used (Table 1). Each horse was administered a single dose (2 mg/kg of body weight) of oseltamivir (Tamiflu™ dry syrup 3%) with 2,000 ml of water via nasogastric tube. Blood samples were collected from a catheter placed in a left jugular vein of each horse with evacuated tubes containing sodium heparin (Venoject™II, Terumo, Tokyo, Japan) at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 hr after administration. Plasma samples were immediately separated from blood cells by centrifugation at 1,000 × g for 10 min (4°C). Then, they were stored at −20°C until assay as described below. The horses were foddered once a day 2 hr before administration of oseltamivir and were supplied with water and hay ad libitum throughout the experiments. In addition, to evaluate the safety of oseltamivir in horses, three horses were orally administered multiple doses of oseltamivir [6 mg/kg, twice a day (12 hr intervals) for five days] using 100 ml plastic syringes on days 7 (horse number 4) and 14 (horse numbers 5 and 6) after the previous experiment. Clinical observations and collection of serum samples for biochemi-

Fig. 1. The structures of oseltamivir, oseltamivir carboxylate (active metabolite, OC) and active metabolite methyl ester. Active metabolite methyl ester was used as the internal standard for the assays of oseltamivir and OC in this study.
tions were conducted daily throughout the experiment. Moreover, to determine the concentration of oseltamivir and OC, plasma samples were obtained from 2 horses (horse numbers 5 and 6) 12 hr after first administration of oseltamivir. All experimental procedures in this study were approved by the Animal Care Committee of the Equine Research Institute.

The standards of oseltamivir and OC (Fig. 1) were gained by the method described in our previous paper [9]. The internal standard (active metabolite methyl ester, I.S.) used in this study (Fig. 1) was gained by methylation of OC. Oseltamivir and OC in plasma were extracted by the method of Wilshire et al. [8]. Briefly, 100 µl of I.S. solution (100 ng/ml) was added to samples (100 µl) and then extracted with solid phase extraction disc cartridges (Empore™ Mixed Phase Cation-MPC, 7 mm/3 m, GL Sciences, Tokyo, Japan). After the elements were evaporated with nitrogen, the dried extracts were reconstituted in 100 µl of deionised water. Reconstituted samples (20 µl) were introduced into a liquid chromatograph mass spectrometer equipped with an atmospheric pressure chemical ionization probe (LCMS-2010A, Shimadzu Corporation, Kyoto, Japan). The two compounds were separated by reversed-phase high performance liquid chromatography (HPLC) using a column (Inertsil ODS-SP, 4.6 × 150 mm, 5 µm, GL Sciences, Tokyo, Japan) at 40°C. The mobile phase was 50% methanol-50% 80 mM aqueous formic acid (1:1, v/v). The flow-rate was 0.2 ml/min, and the retention times for oseltamivir, OC, and I.S. were approximately 3.5, 5.0, and 4.0 min, respectively. The mass spectrometer was operated in positive mode. Quantification was performed using selected ion monitoring of m/z 285 for oseltamivir, m/z 313 for OC, and m/z 299 for I.S., respectively. The quantification limits for both oseltamivir and OC in plasma were 5 ng/ml in this assay. The recovery rates [% mean ± coefficient of variation (CV), n=4] of oseltamivir, OC, and I.S. at 100 ng/ml each were 46.4 ± 0.4, 60.8 ± 1.8, and 80.1 ± 3.3, respectively. Interday CV values for oseltamivir ranged from 0.4 to 9.6% (3 days 4 determinations/day). Interday CV values for OC ranged from 1.8 to 8.1% (3 days 4 determinations/day).

In addition, to determine the binding rate of OC to horse plasma proteins by the ultrafiltration method, plasma was obtained from a pooled sample collected from the above-mentioned six horses 0 hr after administration. OC was added to 500 µl portions of the pooled plasma sample to obtain 2 samples of 100 ng/ml. After incubation for 15 min at 37°C, one sample was filtered by centrifugation at 3,600 × g through filters [ARTKISS™ (molecular weight cut-off: 10,000), Advantec, Tokyo, Japan]. The other sample was untreated. The amounts of OC in the filtrate and untreated samples were measured by HPLC/mass spectrometry, and then the binding rates (%) were calculated. The resulting binding rates were <1%.

The absorption rate constant (Ka) of oseltamivir, maximum concentration in plasma (C max), time to C max (T max), elimination half-life (t1/2), and area under the plasma concentration-versus-time curve (AUC) after administration (AUC 0–∞) for each compound were calculated using one compartment model and the SAAMII software (version 1.2.1, Saam Institute, University of Washington, WA, U.S.A.). The values are shown in Table 2. Mean plasma concentration-time profiles for oseltamivir and OC following administration of oseltamivir are shown in Fig. 2.

The Ka of oseltamivir was 4.1 ± 2.1 hr⁻¹, showing rapid absorption of oseltamivir after oral administration to the horses. The C max of OC in this study (257.9 ± 47.1 ng/ml) was 1.4-fold higher or more than those of 1–2- and 3–5-year-old children reported by Oo et al. (121 and 179 ng/ml, respectively) [5]. Moreover, the T max of OC in this study (1.7 hr) was at least 2.9-fold shorter than those of 1–2- and 3–5-year-old children reported by the same authors (5.0 and 5.6 hr, respectively) [5]. These results suggest that oral administration of oseltamivir would provide higher concentration of OC more rapidly in horses than in children. However, the t1/2 for OC of this study (2.5 hr) was more than 4.5-fold shorter than those of 1–2- and 3–5-year-old children (14.9 and 11.3 hr, respectively) [5], suggesting that OC is by far more rapidly eliminated in horses than in children. Subsequently, the AUC 0–∞ of OC for the horses (1490.6 ng•hr/ml) was approximately twofold less than those of 1–2- and 3–5-year-old children (2810 and 3350 ng•hr/ml, respectively) [5].

We previously reported that OC inhibited the NA activities of EIV strains with 50% inhibitory concentrations (IC 50) ranging from 4.8 to 36.9 ng/ml and the plaque-forming of EIV strains in MDCK cells with 50% effective concentrations (EC 50) ranging from 4.3 to 27.5 ng/ml, except for one resistant strain which had an EC 50 of 3785.1 ng/ml [9]. Since it is known that the binding rate of OC to human plasma proteins is negligible (<3%) and that OC is a time-dependent inhibitor of viral NA and replication [3, 4], the dosage regimen of oseltamivir for humans is therefore designed to retain the trough OC concentration in plasma

### Table 1. Profiles of the experimental horses

<table>
<thead>
<tr>
<th>Horse number</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (month)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anglo-Arb</td>
<td>Female</td>
<td>15</td>
<td>291</td>
</tr>
<tr>
<td>2</td>
<td>Anglo-Arb</td>
<td>Female</td>
<td>13</td>
<td>301</td>
</tr>
<tr>
<td>3</td>
<td>Thoroughbred</td>
<td>Female</td>
<td>15</td>
<td>329</td>
</tr>
<tr>
<td>4</td>
<td>Thoroughbred</td>
<td>Male</td>
<td>13</td>
<td>303</td>
</tr>
<tr>
<td>5</td>
<td>Thoroughbred</td>
<td>Female</td>
<td>12</td>
<td>291</td>
</tr>
<tr>
<td>6</td>
<td>Thoroughbred</td>
<td>Male</td>
<td>12</td>
<td>304</td>
</tr>
</tbody>
</table>
above IC$_{50}$ (and/or EC$_{50}$) as determined in vitro [3]. Likewise, since the binding rate of OC to horse plasma proteins was negligible in this study, it would be possible to design a dosage regimen of oseltamivir for horses on the basis of the trough OC concentration in plasma and IC$_{50}$ (and/or EC$_{50}$).

Actually, in our previous study which investigated the efficacy of oseltamivir in horses experimentally inoculated with A/equine/La Plata/93 (IC$_{50}$, 8.8 $\mu$g/ml; EC$_{50}$, 20.7 $\mu$g/ml) [9, 10], therapeutic administration of oseltamivir (2 mg/kg, at 12 hr intervals for five days from the onset of pyrexia) showed apparent efficacies including rapid reduction of febrile response and disappearance of virus excretion from nostrils. Since the concentration of OC in this pharmacokinetic study 12 hr after administration was 26.6 $\mu$g/ml (Fig. 2), it seemed that concentrations of OC retained above the levels of both IC$_{50}$ and EC$_{50}$ of A/equine/La Plata/93 contributed to the apparent efficacies in the previous study [10]. In contrast, prophylactic administration of oseltamivir (2 mg/kg, at 24 hr intervals for five days from day before viral inoculation) could not prevent the onset of pyrexia and virus excretion from nostrils in the same study [10]. Since the concentration of OC in this study dropped below the EC$_{50}$ of A/equine/La Plata/93 within 18 hr (13.8 $\mu$g/ml) after administration (Fig. 2), the failure of prophylaxis in the previous study [10] was likely due to the concentration of OC being below EC$_{50}$ for 6 hr or more. Whether IC$_{50}$ or EC$_{50}$ as determined in vitro correlates more closely with the efficacy of OC against EIV in horses has not been established at present. Therefore, it would be safer for veterinarians to retain the concentration of OC above 36.9 $\mu$g/ml, which was the highest value for the IC$_{50}$s and EC$_{50}$s of EIVs as determined in vitro [9].

We did not identify any relevant clinical changes [behavior, heart rate, respiration rate, appetite, feces, serum biochemistry values (aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, lactate dehydrogenase, blood urea nitrogen, and creatinine)] in the horses administered multiple high doses of oseltamivir (6 mg/kg at 12 hr intervals for 5 days) throughout the course of the experiment (data not shown). This suggests that oral administration of oseltamivir to horses is well tolerated at single doses of up to 6 mg/kg for five days at 12 hr intervals. Further...

**Table 2.** Pharmacokinetic parameters$^a$ of oseltamivir and oseltamivir carboxylate (active metabolite) in horses

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ka (hr$^{-1}$)</th>
<th>C$_{\text{max}}$ (ng/ml)</th>
<th>T$_{\text{max}}$ (hr)</th>
<th>t1/2 (hr)</th>
<th>AU/C$_{\text{max}}$ (ng•hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oseltamivir</td>
<td>4.1 ± 2.1</td>
<td>149.7 ± 26.9</td>
<td>0.8 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>516.4 ± 76.9</td>
</tr>
<tr>
<td>Oseltamivir carboxylate</td>
<td>ND$^b$</td>
<td>257.9 ± 47.1</td>
<td>1.7 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>1490.6 ± 196.8</td>
</tr>
</tbody>
</table>

$a$) All parameters are represented as means ± standard deviations (n=6). $b$) Not determined.
thermore, the OC concentrations of two horses (horse numbers 5 and 6) 12 hr after first administration of oseltamivir (6 mg/kg) were 87.0 and 69.0 ng/ml, respectively. It is therefore possible that an increase in dose would be an alternative method of retaining the concentration of OC above 36.9 ng/ml with administration intervals of 12 or 24 hr. Further pharmacokinetic investigations in horses are needed to determine the dose of oseltamivir that retains a suitable concentration at 12 or 24 hr after administration.

In summary, this study showed the pharmacokinetics of oseltamivir and OC in horses after oral administration of oseltamivir. The binding of OC to horse plasma proteins was negligible. Oral administration of oseltamivir at 2 mg/kg to horses rapidly provided a concentration of OC in plasma above those necessary to inhibit the NA activities and replications of EIVs. However, because OC in the plasma of the horse is rapidly eliminated, it is suggested that the administration intervals should be less than 10 hr to retain a suitable concentration when using a single dose of 2 mg/kg oseltamivir. These findings will be helpful in designing a rational dosage regimen for oseltamivir for horses.

REFERENCES