Pharmacokinetics of Penciclovir after Oral Administration of its Prodrug Famciclovir to Horses

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(Received 10 August 2009/Accepted 29 October 2009/Published online in J-STAGE 4 December 2009)

ABSTRACT. We investigated the pharmacokinetics of penciclovir after oral administration of its prodrug famciclovir to horses. Following an oral dose of famciclovir at 20 mg/kg, maximum plasma concentrations of penciclovir occurred between 0.75 and 1.5 hr (mean 0.94 ± 0.38 hr) after dosing and were in the range 2.22 to 3.56 μg/ml (mean 2.87 ± 0.61 μg/ml). The concentrations of penciclovir declined in a biphasic manner after the peak concentration was attained. The mean half-life of the rapid elimination phase was 1.73 ± 0.34 hr whereas that of the slow elimination phase was 34.34 ± 13.93 hr. These pharmacokinetic profiles observed were similar to those of another antiviral drug, acyclovir, previously reported in horses following oral dosing of its prodrug valacyclovir.

KEY WORDS: equine, famciclovir, penciclovir, pharmacokinetics.

Equine herpesvirus type 1 (EHV-1) is a major causative agent of respiratory disease in horses and can also lead to abortion and neurological disease [1]. In particular, the recent increase in neurological EHV-1 outbreaks among horses in the U.S.A., the U.K. and Europe is of major concern to the horse industry worldwide, since affected animals often have a poor prognosis [7, 8, 17–20]. The antiviral drug acyclovir has been used for the treatment of EHV-1-associated neurological disorders [13, 20, 36]. Oral administration is preferable to intravenous infusion during outbreaks, but pharmacokinetic studies have revealed that acyclovir given orally to horses is poorly bioavailable and reaches low plasma concentrations [4, 15, 35] that do not exceed the 50% effective concentration (EC50) against EHV-1 strains, as determined by in vitro plaque reduction assay [16]. In contrast, oral administration of valacyclovir, the prodrug of acyclovir, achieves concentrations within the sensitivity range of EHV-1 [15, 23]. Therefore, valacyclovir is now considered to be an attractive candidate for treatment of EHV-1-associated neurological disorders, even though its therapeutic efficacy has not been proven in a controlled study.

Another antiviral drug, famciclovir, is the oral prodrug of penciclovir. Like acyclovir, penciclovir is a guanine analogue. It is phosphorylated to penciclovir monophosphate by the viral thymidine kinase, and then to penciclovir diphosphate and triphosphate by cellular enzymes in virus-infected cells [32, 33]. Penciclovir triphosphate inhibits viral DNA synthesis through competitive inhibition of DNA polymerase; consequently, viral replication in herpesvirus-infected cells is inhibited, with no effect on DNA synthesis in uninfected cells [10, 32]. However, like acyclovir, penciclovir is very poorly absorbed when administered orally to rodents [6, 34] and humans [5]. Famciclovir is the diacetyl ester of 6-deoxy penciclovir and has good bioavailability in humans [5, 12, 24, 34]. After oral administration, famciclovir is converted to penciclovir through di-deacetylation in the intestinal wall and liver and oxidation in the liver [31, 34]. In human medicine, famciclovir is widely used for the treatment of herpes zoster, genital herpes, and orolabial herpes [27]. On the other hand, the drug has not been tested for veterinary use, with the exception of therapy for feline herpesvirus type 1 (FHV-1)-associated disease in cats [22]. EHV-1 is sensitive to penciclovir in vitro, with an EC50 value of 1.64 μg/ml [9] not very different from that of acyclovir (1.7 to 3.0 μg/ml) [16]. This fact suggests that famciclovir could be another option for the treatment of EHV-1-associated neurological disorders, but there are no specific pharmacokinetic data for this compound in horses. Therefore, we investigated the pharmacokinetics of penciclovir after oral administration of famciclovir to horses.

Four healthy adult Thoroughbred horses were used (Table 1). The horses were weighed on the day before drug administration. They were fasted for approximately 18 hr before and 4 hr after drug administration; thereafter, they were supplied with water and hay ad libitum throughout the experiment. Each horse was given a single dose (20 mg/kg body weight) of famciclovir (Famvir Tablets, 250 mg; Maruho, Osaka, Japan) intragastrically. The calculated doses of crushed famciclovir tablets were suspended in 500 ml of water and administered through a stomach tube.

Table 1. Profiles of the experimental horses

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thoroughbred</td>
<td>Female</td>
<td>11</td>
<td>480</td>
</tr>
<tr>
<td>2</td>
<td>Thoroughbred</td>
<td>Female</td>
<td>9</td>
<td>482</td>
</tr>
<tr>
<td>3</td>
<td>Thoroughbred</td>
<td>Female</td>
<td>9</td>
<td>536</td>
</tr>
<tr>
<td>4</td>
<td>Thoroughbred</td>
<td>Female</td>
<td>19</td>
<td>555</td>
</tr>
</tbody>
</table>

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ml of water and administered via a nasogastric tube. The nasogastric tube was flushed with 2000 ml of water immediately after drug administration. A catheter was placed in the left jugular vein of each horse and blood samples were collected in evacuated tubes containing ethylendiaminetetraacetic acid dipotassium salt dihydrate (Venoject II, Terumo, Tokyo, Japan) just before famciclovir administration (baseline sample; time 0), as well as 10, 15, 30, 45, 60, and 90 min and 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, and 30 hr following administration. Plasma samples were immediately separated from blood cells by centrifugation at 2150 × g for 10 min (4°C). Thereafter, they were stored at −20°C until assay, as described below. All experimental procedures were approved by the Animal Care Committee of the Equine Research Institute, Japan.

Penciclovir (LKT Laboratories, Inc., St. Paul, MN, U.S.A.) and the internal standard (I.S.), Penciclovir-d4 (Toronto Research Chemicals Inc., North York, Canada) were commercially obtained. Plasma samples were prepared according to the method published by Schenkel et al. [26]. Briefly, 50 μl of I.S. solution (10 μg/ml) was added to 0.5 ml of plasma sample. Following addition of 2 ml of 0.1 M hydrochloric acid (HCl), the samples were vortexed vigorously for 15 sec, then were loaded onto Oasis MCX 3 cc/60 mg extraction cartridges (Waters, Milford, MA, U.S.A.). After being washed with 2 ml of 0.1 M HCl and 2 ml of methanol, the cartridges were dried under vacuum. Compounds of interest were eluted with a mixture of methanol and 25% ammonia solution (95.5: v/v). After evaporation under a stream of nitrogen at 40°C, dried extracts were reconstituted in 0.5 ml of distilled water. Reconstituted samples (10 μl) were introduced into a liquid chromatography–mass spectrometer equipped with an atmospheric pressure chemical ionization probe (LCMS-2010A, Shimadzu Corporation, Kyoto, Japan). Separation of the penciclovir and I.S. was performed at 40°C on an analytical column (XBridge Shield C18, 2.1 × 150 mm, 2.5 μm; Waters) coupled with a guard column (XBridge Shield C18, 2.1 × 10 mm, 2.5 μm; Waters). The mobile phase was composed of a solvent mixture of 0.1% formic acid – 0.01% nonafluoropentanoic acid as solvent A and acetonitrile as solvent B (A:B=0.17:0.03). A flow rate of 0.2 ml/min was used for sample analysis. The mass spectrometer was operated in positive mode. Quantification was performed using selected ion monitoring of m/z 254 for penciclovir and m/z 258 for I.S. The concentration of penciclovir in each sample was calculated by use of the I.S. method via the peak area ratio and linear regression analysis. Standard curves prepared for the analysis of penciclovir over the concentration ranges of 50 to 2000 ng/ml had correlation factors of 0.999, and the limit of quantification (LOQ) was 50 ng/ml. The recovery rates [% mean ± intraday coefficient of variation (CV), n=5] of penciclovir at 100 ng/ml and 1000 ng/ml, and of I.S. at 1000 ng/ml, were 99.8 ± 5.4, 90.2 ± 6.9, and 88.4 ± 1.9, respectively. Interday CV values for penciclovir at 100 ng/ml and 1000 ng/ml, and of I.S. at 1000 ng/ml, were 9.9%, 6.6%, and 2.7% (3 days, 5 determinations/day), respectively.

Plasma samples that had been taken from 4 untreated healthy horses and stored at −20°C in the Laboratory of Racing Chemistry, Japan were used to determine the rate of binding of penciclovir to horse plasma proteins. Plasma samples were spiked with 0.1 or 2 μg/ml penciclovir. Two samples of each concentration were incubated at 37°C for 30 min, and then one sample was filtered by centrifugation at 1921 × g through filters [Centricut Mini (molecular weight cut-off: 20000), Kurabo, Osaka, Japan]. The other sample was untreated. The amounts of penciclovir in the filtrate and untreated samples were measured by liquid chromatography–mass spectrometry, and then the binding rates (%) were calculated. The means and standard deviations of the plasma protein binding percentage of penciclovir at concentrations of 0.1 and 2 μg/ml in 4 individual horses were 7.45% ± 3.2% and 5.28% ± 2.3%, respectively. The results indicated that penciclovir is poorly bound to horse plasma proteins. Protein binding of penciclovir is also low in the plasma of humans (<16%), rats (<24%), and dogs (<22%) [11, 12].

Plasma drug concentration-time data were assessed via noncompartmental analysis. The maximum plasma concentration (Cmax) of penciclovir and time to Cmax (Tmax) were estimated directly from the data. The areas under the plasma concentration versus time curves (AUC) between zero and the last data point (AUCt) were determined by a linear trapezoidal method. The AUC between the last data point and infinity (AUCt-inf) was calculated by dividing the final observed concentration by the rate constant of the terminal phase. The AUC between zero and infinity (AUC0-inf) was estimated by summing AUC0-t and AUCt-inf. The elimination rate constant (λ) was calculated by linear regression analysis, using at least 3 data points of the log concentration time plots. The elimination half-life (t1/2) of penciclovir was calculated as the natural logarithm of 2 divided by the λ value. The values are shown in Table 2. The mean plasma concentration-time profile of penciclovir after oral administration of famciclovir is shown in Fig. 1.

No adverse effects were noted in any of the 4 horses during this trial. Following an oral dose of penciclovir at 20 mg/kg, maximum plasma concentrations of penciclovir occurred between 0.75 and 1.5 hr (mean 0.94 ± 0.38 hr) after dosing and were in the range 2.22 to 3.56 μg/ml (mean 2.87 ± 0.61 μg/ml). The concentrations of penciclovir declined rapidly from Tmax until 8 hr post dose (rapid elimination phase), but declined very slowly thereafter (slow elimination phase). The mean half-life of the rapid elimination phase (t1/2,1) was 1.73 ± 0.34 hr, whereas that of the slow elimination phase (t1/2,2) was 34.34 ± 13.93 hr. The mean AUC0-t and AUC0-inf were 8.38 ± 0.44 and 13.08 ± 1.59 μg/hr/ml, respectively.

It has been reported that little or no famciclovir was detected in the plasma of human adults, rats and dogs after oral administration, which suggests that substantial first-pass metabolism of famciclovir occurs in those animals [11, 12]. Similarly, in our preliminary study using a horse, fam-
Penciclovir was not detected in the plasma following dosing orally at 15 mg/kg (data not shown). This result suggests that famciclovir might also undergo substantial first-pass metabolism in horses. Therefore, we have only measured concentrations of penciclovir in this study. Like in horses, the peak plasma concentration of penciclovir occurs rapidly in human adults (0.75 hr) and rats (0.5 hr) following oral dosing of famciclovir [11, 25]. In contrast, the T_{\text{max}} observed in dogs (3 hr) is relatively long [11]. The penciclovir C_{\text{max}} values observed in human adults (5.09 ± 0.99 μg/ml), rats (3.5 ± 0.2 μg/ml), and dogs (4.4 ± 0.6 μg/ml) are not very different from that in horses, although the dosage of famciclovir differed among studies (human adults: approximately 10 mg/kg, rats: 40 mg/kg, and dogs: 25 mg/kg) [11, 25]. The observed slow elimination phase, which is not likely to be seen in human adults, rats, or dogs, might have been due to our use of a sensitive method of penciclovir detection (LOQ: 50 ng/ml). If an LOQ of 200 ng/ml, which was used for studies for other species, were applied to our results, then the penciclovir plasma concentration would fall below the LOQ at 8 to 10 hr post dose. The mean half-life of the rapid elimination phase (t_{1/2a} = 1.73 hr) observed here was similar to the elimination half-life in human adults (2.16 hr), which is a little shorter than that in dogs (2.8 hr) and longer than that in rats (0.7 hr) [11, 25]. These results suggested that the pharmacokinetics of penciclovir in horses following oral dosing of famciclovir are similar to those in human adults, although the reduced C_{\text{max}} observed here indicated that the bioavailability in horses would likely be lower than that in humans. In contrast, the pharmacokinetics of penciclovir in cats following oral administration of famciclovir are quite different from those in other species [30], although famciclovir has been used to treat FHV-1-associated disease in cats [22]. The C_{\text{max}} of penciclovir is reached slowly, 4.6 hr after a single dosing of famciclovir (15 mg/kg), and its mean value is only 0.35 ± 0.18 μg/ml, which is notably lower than the EC_{50} value for FHV-1 (3.5 μg/ml) [21]. On the other hand, the values of C_{\text{max}} (range 2.22 to 3.56 μg/ml) that we observed exceeded the EC_{50} values for EHV-1 (1.64 μg/ml) [9]. Plasma penciclovir concentrations could be maintained above the EC_{50} values for approximately 1 hr.

Following oral administration of valacyclovir to horses at 20 mg/kg, the total plasma concentration of acyclovir, the active metabolite of valacyclovir, rapidly increased, with a C_{\text{max}} of 4.16 ± 1.42 μg/ml at 0.90 ± 0.18 hr [15]. The mean elimination half-life was 2.03 hr and the mean AUC_{0-\infty} was 10.0 ± 3.68 μg•hr/ml [15]. These pharmacokinetic parameters were similar to the ones we observed following oral administration of famciclovir, although the valacyclovir study did not report a slow elimination phase after dosing. However, the slow elimination of plasma acyclovir concentration after oral administration of valacyclovir to horses has been reported by another study [23]. Although most of experimental conditions, including drug dose, LOQ of acyclovir assay, and duration of sampling period, were almost the same between the 2 valacyclovir studies, the latter used older horses (9 ± 3 years) than the former (3.2 ± 1.3 years), and this might have affected the drug elimination profile. The proposed rational dosage regimens for valacyclovir treatment for horses were (i) a dose of 40 mg/kg every 8 hr [15] or (ii) loading doses of 27 mg/kg every 8 hr for 2 days, followed by a maintenance dose of 18 mg/kg every 12 hr [23]. These regimens were designed to make it possible to maintain plasma acyclovir concentrations above the EC_{50} values of EHV-1 (1.7 to 3.0 μg/ml) [16] during the majority of the dosing interval. This was based on a report that max-

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**Table 2. Pharmacokinetic parameters of penciclovir in horses**

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>C_{\text{max}} (μg/ml)</th>
<th>T_{\text{max}} (hr)</th>
<th>t_{1/2a} (hr)</th>
<th>t_{1/2b} (hr)</th>
<th>AUC_{0-\text{t}} (μg•hr/ml)</th>
<th>AUC_{0-\text{inf}} (μg•hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.53</td>
<td>1.50</td>
<td>1.58</td>
<td>54.88</td>
<td>7.78</td>
<td>15.34</td>
</tr>
<tr>
<td>2</td>
<td>2.22</td>
<td>0.75</td>
<td>2.23</td>
<td>27.11</td>
<td>8.60</td>
<td>12.71</td>
</tr>
<tr>
<td>3</td>
<td>3.17</td>
<td>0.75</td>
<td>1.63</td>
<td>30.79</td>
<td>8.32</td>
<td>12.65</td>
</tr>
<tr>
<td>4</td>
<td>3.56</td>
<td>0.75</td>
<td>1.48</td>
<td>24.56</td>
<td>8.80</td>
<td>11.61</td>
</tr>
<tr>
<td>Mean</td>
<td>2.87</td>
<td>0.94</td>
<td>1.73</td>
<td>34.34</td>
<td>8.38</td>
<td>13.08</td>
</tr>
<tr>
<td>Standard deviations</td>
<td>0.61</td>
<td>0.38</td>
<td>0.34</td>
<td>13.93</td>
<td>0.44</td>
<td>1.59</td>
</tr>
</tbody>
</table>

C_{\text{max}}, maximum plasma drug concentration; T_{\text{max}}, time to C_{\text{max}}; t_{1/2a}, half-life of rapid elimination phase; t_{1/2b}, half-life of slow elimination phase; AUC_{0-\text{t}}, area under the plasma drug concentration versus time curve between zero and the last data point; AUC_{0-\text{inf}}, AUC between zero and infinity.
imum efficacy in the treatment of herpes simplex virus infection in humans is reached when the acyclovir concentration remains above the EC50 for longer than 12 hr in each 24-hr period [29].

The similarity of the pharmacokinetics and pharmacodynamics of penciclovir to those of acyclovir suggests that the dosage regimens for valacyclovir therapy against EHV-1 mentioned above could apply to famciclovir therapy. However, recently, in a controlled study, no therapeutic effect of valacyclovir against EHV-1-related respiratory disease was seen, even though sufficiently high acyclovir concentrations (above the EC50) were maintained in plasma via a dose of 40 mg/kg every 8 hr for 5 or 7 consecutive days [14]. Therefore, higher doses and/or increased administration frequency of valacyclovir or famciclovir would be required for efficient treatment for EHV-1 infection. It has been reported that the intracellular half-lives of penciclovir triphosphate are much longer than those of acyclovir triphosphate in human cells infected with herpes simplex virus type 1 (HSV-1), type 2 (HSV-2), or varicella-zoster virus (VZV) (10 hr vs. 0.7 hr, 20 hr vs. 1 hr, and 9.1 hr vs. 0.8 hr, respectively) [3, 10, 33]. Additionally, in both tissue culture [2] and animal infection [6, 28], penciclovir has a more sustained antiviral effect than acyclovir against HSV-1 and -2, a finding that may be correlated with the difference in the stability of the 2 compounds in infected cells. If penciclovir triphosphate is more stable than acyclovir triphosphate in EHV-1-infected cells as well, then it might be possible to obtain therapeutic efficacy of famciclovir via a dosage regimen using lower dose than that required for valacyclovir therapy.

In summary, oral administration of famciclovir at 20 mg/kg to horses rapidly provided plasma penciclovir concentrations within the sensitivity range of EHV-1. The pharmacokinetic parameters observed were similar to those reported previously in horses following oral dosing of valacyclovir. These results should encourage further research efforts to test the therapeutic efficacy of famciclovir against EHV-1 in a controlled study.

ACKNOWLEDGMENTS. We thank Akira Kokubun, Kazue Arakawa, and Miki Kuramochi for their invaluable technical assistance. We also thank all the staff members of Equine Research Institute who kept the horses in good condition.

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