Pharmacokinetics of Acyclovir after Intravenous Infusion of Acyclovir and after Oral Administration of Acyclovir and Its Prodrug Valacyclovir in Healthy Adult Horses

B. Garré,1,2 K. Shebany,1 A. Gryspeert,2 K. Baert,1 K. van der Meulen,2 H. Nauwynck,2 P. Deprez,3 P. De Backer,1 and S. Croubels1*

Department of Pharmacology, Toxicology, Biochemistry, and Organ Physiology,1 Laboratory of Virology,2 and Department of Internal Medicine and Clinical Biology of Large Animals,3 Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Received 26 January 2007/Returned for modification 2 July 2007/Accepted 2 September 2007

The purpose of this study was twofold. The first aim was to evaluate the oral bioavailability and pharmacokinetics (PKs) of acyclovir in horses after intravenous (i.v.) administration and after oral administration of acyclovir and its prodrug, valacyclovir. Second, we aimed to combine these PK data with pharmacodynamic (PD) information, i.e., 50% effective concentrations (EC50 values) from in vitro studies, to design an optimal dosage schedule. Three treatments were administered to healthy adult horses: 10 mg of acyclovir/kg of body weight delivered as an i.v. infusion over 1 h, 20 mg of acyclovir/kg administered as tablets by nasogastric intubation, and 20 mg of valacyclovir/kg administered as tablets by nasogastric intubation. Total plasma concentrations were measured by a high-performance liquid chromatography method combined with fluorescence detection, while unbound plasma concentrations were determined by liquid chromatography-tandem mass spectrometry. The peak concentration of i.v. acyclovir was approximately 10 μg/ml for both the total and the unbound plasma concentrations. The mean half-life of elimination was between 5.05 h (total concentration) and 11.9 h (unbound concentration). Oral administration of acyclovir resulted in low maximum concentration in plasma (Cmax) and poor bioavailability. A 10-times-higher Cmax and an 8-times-higher bioavailability were achieved with oral administration of valacyclovir. The i.v. administration of 10 mg/kg acyclovir and the oral administration of 20 mg/kg valacyclovir achieved concentrations within the sensitivity range of equine herpesvirus type 1 (EHV-1). The higher bioavailability of valacyclovir makes it an attractive candidate for the prophylactic and/or therapeutic treatment of horses infected with EHV-1. The results from the PK/PD modeling showed that a dosage of 40 mg/kg valacyclovir, administered three times daily, would be sufficient to reach plasma concentrations above the EC50 values.

Equine herpesvirus type 1 (EHV-1), a member of the subfamily Alphaherpesvirinae, is a highly prevalent equine pathogen. The virus is endemic worldwide, and most horses become infected during their first year of life (21). EHV-1 can cause abortion or neonatal foal death (1, 6) and, occasionally, neurological damage, resulting in paralysis. Neurological disorders have been reported with increasing frequency (10, 17, 24, 27, 28). During an outbreak, many cases may occur, with devastating effects. Several recent outbreaks of EHV-1 myeloencephalopathy have raised awareness of the disease within the veterinary community and by the public at large (14, 23, 29). Although vaccines are available, they are not fully protective, and outbreaks may occur even in vaccinated herds. Therefore, there is a need for antiviral therapy.

A recent study investigated the in vitro susceptibilities of six isolates of EHV-1 to several antivirals, i.e., acyclovir, ganciclovir,cidofovir, adefoxiv, 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine, and foscarnet (12). On the basis of the 50% effective concentration (EC50) for the inhibition of plaque formation, ganciclovir emerged as the most potent compound against all six isolates. Acyclovir was approximately 10-fold less effective (EC50, 1.7 to 3.0 μg/ml). However, due to reported hematotoxicity in humans (2), the specific indications for cytomegalovirus infections in humans, and the high cost of ganciclovir, acyclovir seems to be a more attractive candidate for antiviral therapy against EHV-1 infection in horses.

Several recent reports have described the use of the antiviral drug acyclovir for the treatment of neurological disorders (14, 27, 29), as it has been used for the management of the neonatal form of EHV-1 infection (19). The benefit of these treatments is difficult to evaluate since no untreated control animals were included in the studies. The dosages used in those studies were extrapolated from the dosages recommended in human medicine and were not based on the pharmacokinetic (PK) properties of acyclovir in the horse.

Recently, the PKs of acyclovir after intravenous (i.v.) and oral (p.o.) administration have been determined in horses (5, 30). The i.v. administration of acyclovir achieved plasma concentrations within the sensitivity range described for EHV-1. For practical application during an outbreak, a p.o. form rather than repeated i.v. infusion would be preferred. However, the p.o. administration of acyclovir resulted in low bioavailability.
and low plasma levels, so a therapeutic benefit may not be expected from this treatment. Higher levels may be achieved with the oral prodrug of acyclovir, valacyclovir, as it has a higher bioavailability in humans. In humans, valacyclovir has been found to have a three to five times higher bioavailability than acyclovir, i.e., 54% for valacyclovir versus 12 to 20% for acyclovir (20).

The first aim of the present study was to evaluate the oral bioavailability (F) and PKs of total and unbound acyclovir after the i.v. administration of acyclovir and after the p.o. administration of acyclovir and its prodrug, valacyclovir. The unbound concentration of acyclovir may be of particular interest, as this is the concentration that can reach the biophase and that has an effect on viral replication. The second aim was to combine PK and pharmacodynamic (PD) information to design an optimal dosage regimen so that plasma concentrations exceed the EC50 value of EHV-1 during the entire treatment interval. This is based on the reasoning that an antiviral drug should be effective when the plasma level exceeds the EC50 value of the virus determined in vitro (8, 26).

To our knowledge, this is the first time that the PKs and F of valacyclovir have been studied in horses. Moreover, PK/PD approaches have thus far not been applied in veterinary antiviral therapy.

MATERIALS AND METHODS

Animals. Six healthy adult horses (three males, three females) aged 3.2 ± 1.3 years (mean ± standard deviation [SD]) and weighing 446 ± 54 kg were studied by the use of a three-way crossover design with a 1-week washout period between treatments. The horses were weighed on the day before each treatment. They were housed in individual stables, where they had continuous access to hay and water. A single-lumen i.v. catheter for blood sampling (Cavalix Certo; B Braun, Diegem, Belgium) was aseptically placed in one jugular vein prior to the start of the study. For the i.v. study, a short i.v. catheter (Intraflon 2; Vygon, Brussels, Belgium) was placed in the contralateral jugular vein for 1 h for acyclovir administration. For the p.o. treatments, the horses were fasted for 12 h before and 4 h after administration. All procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University (EC2005/44).

Drug administration and blood sampling. Treatments consisted of the i.v. administration of acyclovir at a dosage of 10 mg/kg of body weight and acyclovir or valacyclovir at 20 mg/kg intragastrically (p.o.). All the products were kindly donated by GlaxoSmithKline (Genva, Belgium). The calculated dose of injectable acyclovir (Zovirax I.V., acyclovir for injection, 250 mg) was added to isotonic saline in a total amount of 300 ml and was given through the short i.v. catheter by constant-rate infusion with a fluid pump (IVAC Star-Flow 580; IVAC Corporation, San Diego, CA) over 1 h. The p.o. doses were prepared identically for all horses by suspending crushed acyclovir tablets (Zovirax 200, acyclovir tablets, 200 mg) or valacyclovir tablets (Zelitrex 500, valacyclovir tablets, 500 mg) in 100 ml of tap water, followed by administration through a nasogastric tube. The nasogastric tubes were flushed with at least 200 ml of water immediately after administration.

Baseline (time zero) samples were collected just before drug administration. During the i.v. administration of acyclovir, plasma samples were collected at 20, 40, and 60 min (the end of the infusion). Additional samples were obtained at 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 16, 23, 30, and 48 h after the start of the infusion. For the p.o. dosing study, plasma samples were collected at 20, 40, and 60 min and at 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 16, 23, and 30 h postadministration. Samples were collected into heparinized blood collection tubes (Venoject; Terumo, Leuven, Belgium), centrifuged at 10,000 × g for 10 min, the supernatant was transferred to another tube. Next, 5 ml of dichloromethane-isopropyl alcohol (50/50; vol/vol) was added. After vortex mixing for 30 s and then subsequently deproteinized by the addition of 100 μl of pentafluoropropanionic acid. After vortex mixing for 1 min and centrifugation at 10,000 × g for 10 min, the supernatant was transferred to another tube. Next, 5 ml of dichloromethane-isopropyl alcohol (50/50; vol/vol) was added. After vortex mixing for 30 s, the samples were allowed to extract for 10 min by gentle rolling. Then, the samples were centrifuged at 900 × g for 10 min and the organic phase was removed and evaporated to dryness under a stream of nitrogen at 40°C. The residue was reconstituted in 200 μl of mobile phase solvent A (which consisted of 0.05 M heptane sulfonic acid sodium salt in water, with the pH adjusted to 2.5 with a phosphoric acid [85%, by weight] solution), and a volume of 100 μl was injected into the HPLC fluorescence system.

The HPLC system consisted of a P1000XR quaternary gradient pump, an AS3000 autosampler with a device that cooled the sample to 12°C, and a type Jasco FP-920 fluorescence detector, all from Thermo Separation Products (San Jose, CA). Chromatographic conditions were achieved by using an Inertsil 5 ODS-3 column (250 by 4.6 mm [inner diameter], which was kept at room temperature) in combination with a guard column (SS; 10 by 2 mm) from Varian (Middelburg, The Netherlands). The mobile phase consisted of 90% solvent A and 10% solvent B (methanol). Gradient elution was performed at a flow rate of 1 ml/min (0 to 22 min with 90% solvent A and 10% solvent B, 22 to 30 min with 50% solvent A and 50% solvent B, and 30 to 40 min with 90% solvent A and 10% solvent B). The eluate was monitored with fluorescence detection at an excitation wavelength of 270 nm and an emission wavelength of 360 nm.

FIG. 1. Total plasma concentration-versus-time plot (mean ± SD) of acyclovir in six horses during and following a 1-h constant-rate infusion of acyclovir at a dose of 10 mg/kg (●), after p.o. administration of acyclovir at 20 mg/kg (■), and after p.o. administration of valacyclovir at 20 mg/kg (△).

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TABLE 1. PK parameters derived from compartmental analysis for total and unbound acyclovir administered as an i.v. infusion (1 h) to six horses at 10 mg/kg

<table>
<thead>
<tr>
<th>Concn</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$A$ (µg/ml)</th>
<th>$B$ (µg/ml)</th>
<th>$C$ (µg/ml)</th>
<th>$\alpha$ (1/h)</th>
<th>$\beta$ (1/h)</th>
<th>$\gamma$ (1/h)</th>
<th>$t_{1/2a}$ (h)</th>
<th>$t_{1/2b}$ (h)</th>
<th>$t_{1/2c}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>10.7 ± 2.79</td>
<td>29.7 ± 12.8</td>
<td>1.02 ± 0.48</td>
<td>0.53 ± 0.35</td>
<td>3.06 ± 0.52</td>
<td>0.14 ± 0.08</td>
<td>0.23b</td>
<td>5.05b</td>
<td>1.23b</td>
<td>11.9b</td>
</tr>
<tr>
<td>Unbound</td>
<td>10.5 ± 3.98</td>
<td>37.8 ± 13.8</td>
<td>2.37 ± 1.03</td>
<td>0.53 ± 0.35</td>
<td>6.83 ± 6.67</td>
<td>0.56 ± 0.24</td>
<td>0.06 ± 0.05</td>
<td>1.04</td>
<td>1.23b</td>
<td>11.9b</td>
</tr>
</tbody>
</table>

$a$ Values are means ± SDs unless indicated otherwise. Abbreviations: $C_{\text{max}}$, maximal plasma drug concentration; $A$ and $B$, preexponential terms for distribution and elimination phases (two-compartment model); $C$, preexponential terms for rapid distribution, slow distribution, and elimination phases, respectively (three-compartment model); $\alpha$, $\beta$, and $\gamma$, exponential terms for distribution and elimination phases, respectively (two-compartment model); $t_{1/2a}$, $t_{1/2b}$, and $t_{1/2c}$, rapid distribution, slow distribution, and elimination $t_{1/2}$, respectively (three-compartment model); $k_{10}$, $k_{12}$, and $k_{21}$, distribution and elimination microconstants for two-compartment model; $k_{10}$, $k_{12}$, $k_{21}$, $k_{13}$, and $k_{24}$, distribution and elimination microconstants for three-compartment model; $V_{\text{ss}}$, total body clearance; $V_{1/2}$, apparent volume of distribution at steady state; AUC, area under the plasma drug concentration-time curve; MRT, mean residence time.

$^b$ Values are harmonic means.

(ii) Unbound plasma concentration. Two hundred microliters of plasma and 50 µl of the IS solution at 0.1 µg/ml were added to a Microcon YM-30 centrifugal filter device (molecular weight cutoff, 30,000; Millipore, Brussels, Belgium). The samples were vortexed for 30 s and then centrifuged at 10,000 × g for 15 min. An aliquot of 10 µl was injected into the LC-MS/MS instrument.

The HPLC system consisted of a Thermo Surveyor MS pump Plus and a column heater module, both from Thermo Electron Corporation (Waltham, MA). Chromatography was performed with a Nucleodur C18 Pyramid column (125 by 2 mm [inner diameter]; 3 µm), in combination with a precolumn filter (CC 8/3 Nucleodur C18 Pyramid; 3 µm; Macherey-Nagel, Düren, Germany). The temperature of the autosampler was set at 8°C. The mobile phase solvent A was a solution of 5 mM ammonium formate in HPLC water and solvent B was methanol, run at 300 µl/min. Gradient elution was performed (0 to 5.5 min with 95% solvent A and 5% solvent B, 5.5 to 10 min with 30% solvent A and 70% solvent B, and 10 to 19 min with 95% solvent A and 5% solvent B).

The mass spectrometer was a TSQ-Quantum triple-quadrupole instrument from Thermo Electron Corporation equipped with an electrospray ionization source operating in the positive ion mode. The instrument was calibrated with tyrosine 1,3,6 standards, according to the manufacturer’s instructions. Thereafter, the instrument was tuned for acyclovir and ganciclovir (IS) by the direct infusion of a 1-µg/ml solution. The following tune parameters were used for both analytes: spray voltage, 4.8 kV; sheath gas pressure, 49 arbitrary units; auxiliary gas pressure, 4 arbitrary units; and capillary temperature, 350°C.

The instrument was operated in the selected reaction monitoring mode by using a product ion mass-over-charge ratio (m/z) 152.2 for acyclovir and ganciclovir. The selected reaction monitoring transitions at m/z 226 to 152.2 for acyclovir and at m/z 256 to 152.2 for ganciclovir were monitored from 2.5 to 6.5 min.

Both methods for the total and the unbound concentrations were validated according to European Commission guidelines (3). The LOQ was defined as the lowest concentration of acyclovir for which the method was validated with an accuracy and a precision that fell within the recommended ranges. The LOQs were 50 ng/ml and 5 ng/ml for the HPLC fluorescence method and the LC-MS/MS method, respectively.

Plasma protein binding study. Plasma was collected from each horse (horses A to F) before treatment started. Fresh plasma samples were spiked with 0.5, 5, and 15 µg/ml acyclovir. Two samples of each concentration were incubated at 37°C for 1 h and then extracted as described above by using Microcon filters to determine the unbound concentration. The percentage of drug bound to plasma proteins was calculated by the following equation:

$$\text{bound drug} (%) = \left(\frac{\text{total plasma concentration} - \text{unbound plasma concentration}}{\text{total plasma concentration}}\right) \times 100$$

PK analysis. For analysis of drug concentration-time curves, compartmental PK modeling and noncompartmental PK modeling were performed by using a computerized program (WinNonLin, version 5.01; Pharsight, Mountain View, CA). The model with the best fit was selected after visual inspection of the fitted curve and by selection of the model with a smaller value for Akaike’s information criterion (31) and a better correlation coefficient. Samples with concentrations below the LOQ were not included in the data analysis.

The total plasma concentration after i.v. administration was best described by a two-compartment model, while the unbound plasma concentration after i.v. administration was best described by a three-compartmental model. The corresponding equations during and after infusion are as follows:

$$C_t = \sum_{i=1}^{n} \frac{C_i}{k_{i,T}} \cdot \left(1 - e^{-k_{i,T} \cdot t}\right) \quad (t < T)$$

$$C_t = \sum_{i=1}^{n} \frac{C_i}{k_{i,T}} \cdot \left(e^{-k_{i,T} \cdot T} - e^{-k_{i,T} \cdot t}\right) \quad (t \geq T)$$

where $C_i$ is the plasma drug concentration at time $t$; $T$ is the infusion duration, and $k_i$ and $\lambda_i$ are the coefficients and exponents of the equation, respectively (13).

The elimination half-life ($t_{1/2}$) was estimated from the relationship:

$$t_{1/2} = \ln 0.5 / k_{el}$$

where $k_{el}$ represents the elimination rate constant, i.e., $\beta$ and $\gamma$ for the two- and three-compartmental models, respectively.

The area under the curve (AUC) from time $t$ to infinity was calculated by using the linear trapezoidal method for AUC and adding the estimated terminal portion of the curve ($C_{\text{inf}}$, where $r$ is the time of the last measurable plasma concentration).

The data obtained after the p.o. administrations were analyzed by noncompartmental methods (11). The absolute F values for of acyclovir and valacyclovir were determined by the equation:

$$F(\%) = \frac{\text{AUC(i.v.)}}{\text{AUC(p.o.)}} \cdot \frac{\text{dose(i.v.)}}{\text{dose(p.o.)}} \times 100$$

To determine whether the PK parameters differed for total drug and unbound drug, statistical analysis was performed on the basis of analysis of variance with SPSS software (SPSS Inc., Chicago, IL).

PK/PD index and PK/PD breakpoint. The PK/PD index that was used was the time that the concentration remained above the EC50, defined as the cumulative percentage of time over a 24-h period that the drug concentration exceeds the EC50 (4). This is expressed as a percentage of the dosage schedule, which is the PK/PD breakpoint and which should be more than 50% (26).

RESULTS

No adverse effects were observed in any of the six horses. The major reported side effect of parenteral administration of acyclovir in humans is renal toxicity. This is related to the precipitation of acyclovir crystals in renal tubules if the maximum solubility is exceeded or if the drug is given by bolus injection. Therefore, the renal functions of all six horses were monitored. No significant changes in urea and creatinine levels in comparison to the levels at the baseline on day −1 ($P > 0.05$) were seen during this trial.
**Total plasma concentration.** The mean total plasma acyclovir concentration-versus-time curves after i.v. administration of acyclovir and after p.o. dosing of acyclovir and valacyclovir are shown in Fig. 1. Table 1 presents the PK parameters after i.v. administration. The peak concentration ($C_{\text{max}}$) for i.v. acyclovir was 10.7 ± 2.79 μg/ml (mean ± SD). The harmonic mean $t_{1/2}$ of the distribution phase was 0.23 h, while the harmonic mean $t_{1/2}$ of the elimination phase was 5.05 h. The volume of distribution at steady state was 2.96 ± 0.209 liters/kg.

After intragastric administration of acyclovir, F was only 3.13% and the plasma concentrations dropped below the LOQ of the HPLC fluorescence method after 5 h.

Intragastric administration of valacyclovir was associated with an $F$ of 26.1% ± 4.77%. After 0.90 ± 0.18 h, the maximum concentration of 4.16 ± 1.42 μg/ml was reached. The harmonic mean $t_{1/2}$ of the elimination phase was 2.03 h (Table 2).

**Unbound plasma concentration.** The mean unbound plasma acyclovir concentration-versus-time curves after i.v. administration of acyclovir and after p.o. dosing of acyclovir and valacyclovir are shown in Fig. 2. Table 1 presents the PK parameters after i.v. administration. The peak concentration (mean ± SD) for i.v. acyclovir was 10.5 ± 3.98 μg/ml. The harmonic mean $t_{1/2}$ of the rapid distribution phase, the slow distribution phase, and the elimination phase were 0.10 h, 1.23 h, and 11.9 h, respectively. The volume of distribution at steady state was 9.81 ± 11.4 liters/kg.

After intragastric administration of acyclovir, F was only 7.52% ± 2.06%. Plasma acyclovir concentrations remained above the assay’s LOQ for the duration of the sampling period. The maximum concentration was 0.40 ± 0.14 μg/ml at 1.06 ± 0.49 h after dosing.

An $F$ of 26.4% ± 7.11% was obtained after the intragastric administration of valacyclovir. At 0.78 ± 0.17 h, a maximum concentration of 4.76 ± 1.67 μg/ml was reached. The harmonic mean $t_{1/2}$ of the elimination phase was 8.79 h (Table 2).

**Plasma protein binding.** The mean value and SD (n = 6) of the plasma protein binding capacity of acyclovir in pooled plasma from the six horses are presented in Fig. 3A. Levels of binding of between 10 and 20% were calculated. There was a tendency for decreasing plasma protein binding with increasing concentrations. However, there was no significant difference between the various concentrations and the percent binding ($P > 0.05$). The means and SDs of the plasma protein binding for three concentrations (0.5, 5, and 15 μg/ml) for the six individual horses are presented in Fig. 3B. The differences between the horses were not significant ($P > 0.05$).

**PK/PD index.** After i.v. administration of 10 mg/kg of acyclovir and p.o. administration of 20 mg/kg of valacyclovir, the plasma concentrations could be maintained above the EC$_{50}$ value for 1.5 to 2 h. A single dose of 20 mg/kg of acyclovir administered p.o. did not result in plasma concentrations higher than the EC$_{50}$ value. Using the nonparametric superposition tool of WinNonLin software, we designed a dosage schedule for the p.o. administration of valacyclovir that makes it possible to obtain plasma concentrations above the EC$_{50}$ value of EHV-1 during the majority of the dosing interval. Figure 4 shows the predicted acyclovir concentration-versus-time curve based on a dosage schedule of 40 mg/kg of valacyclovir, every 8 h, together with the upper and lower EC$_{50}$ values, i.e., 3.0 μg/ml and 1.7 μg/ml, respectively.

**DISCUSSION**

The present study was conducted to evaluate the $F$ and PKs of total and unbound acyclovir after i.v. administration of acyclovir and after p.o. administration of acyclovir and its prodrug, valacyclovir. Additionally, we wanted to combine PK and PD information to design an optimal dosage regimen for prophylactic and/or therapeutic treatment of horses during an EHV-1 outbreak.

In horses, like in humans, the p.o. administration of valacyclovir is associated with higher bioavailability than the parent drug. An eight-times-higher $F$ was noted for valacyclovir, resulting in plasma concentrations exceeding those which inhibit the plaque formation of EHV-1 in vitro. This strongly indicates the clinical applicability of this drug in cases of EHV-1 infection in horses.

### TABLE 1—Continued

<table>
<thead>
<tr>
<th>$k_{\text{10}}$ (1/h)</th>
<th>$k_{\text{21}}$ (1/h)</th>
<th>$k_{\text{12}}$ (1/h)</th>
<th>$k_{\text{31}}$ (1/h)</th>
<th>$k_{\text{13}}$ (1/h)</th>
<th>$V_{\text{C}}$ (litters/kg)</th>
<th>$AUC$ (μg · h/ml)</th>
<th>MRT (h)</th>
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<tr>
<td>1.72 ± 0.56</td>
<td>0.23 ± 0.11</td>
<td>1.25 ± 0.34</td>
<td>0.10 ± 0.05</td>
<td>2.43 ± 3.12</td>
<td>0.59 ± 0.18</td>
<td>2.96 ± 2.09</td>
<td>8.0 ± 4.25</td>
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<tr>
<td>1.66 ± 0.62</td>
<td>0.88 ± 0.39</td>
<td>2.39 ± 2.87</td>
<td>0.10 ± 0.05</td>
<td>2.43 ± 3.12</td>
<td>0.41 ± 0.04</td>
<td>0.27 ± 0.09</td>
<td>9.81 ± 11.4</td>
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### TABLE 2. PK parameters derived from noncompartmental analysis for total and unbound acyclovir determined after a single 20-mg/kg dose of acyclovir and valacyclovir administered p.o. to six horses

<table>
<thead>
<tr>
<th>Conc</th>
<th>$C_{\text{max}}$ (μg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$t_{\text{1/2}}$ (h)</th>
<th>AUC (μg · h/ml)</th>
<th>$F$ (%)</th>
<th>$C_{\text{max}}$ (μg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$t_{\text{1/2}}$ (h)</th>
<th>AUC (μg · h/ml)</th>
<th>$F$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.33 ± 0.14</td>
<td>1.04 ± 0.26</td>
<td>1.56$^b$</td>
<td>1.16 ± 0.45</td>
<td>3.13 ± 1.21</td>
<td>4.16 ± 1.42</td>
<td>0.90 ± 0.18</td>
<td>2.03$^b$</td>
<td>10.0 ± 3.68</td>
<td>26.1 ± 4.77</td>
</tr>
<tr>
<td>Unbound</td>
<td>0.40 ± 0.14</td>
<td>1.06 ± 0.49</td>
<td>13.5$^b$</td>
<td>3.53 ± 0.87</td>
<td>7.52 ± 2.06</td>
<td>4.76 ± 1.67</td>
<td>0.78 ± 0.17</td>
<td>8.79$^b$</td>
<td>14.9 ± 1.03</td>
<td>26.4 ± 7.11</td>
</tr>
</tbody>
</table>

$^a$ Values are means ± SDs unless indicated otherwise. Abbreviations: $C_{\text{max}}$, maximal plasma drug concentration; $T_{\text{max}}$, time to maximal plasma drug concentration; $t_{\text{1/2}}$, elimination $t_{1/2}$; AUC, area under the plasma drug concentration-time curve; $F$, oral bioavailability.

$^b$ Values are harmonic means.
Similar plasma concentrations were reached for both total and unbound acyclovir. This can be explained by a rather low protein binding, since the free fractions varied between 0.8 and 0.9 in horses (Fig. 3). However, by the LC-MS/MS method, 10-fold lower acyclovir concentrations could be detected compared to those detected by the HPLC fluorescence method. Due to this improved detection sensitivity, a more prolonged elimination phase was observed and a three-compartment model best described the data after i.v. administration. The total body clearance was similar for both total and unbound drug, but the volume of distribution seemed higher for the unbound concentration. As a consequence, the $t_{1/2}$ of elimination seemed longer, i.e., 11.9 and 5.05 h for total and unbound drug, respectively. However, no statistically significant differences could be detected ($P > 0.05$). After the intragastric administration of valacyclovir, maximum concentrations were already reached within 1 h. Again, the mean $t_{1/2}$ of elimination of 8.79 h seemed longer for the unbound concentration, but these differences were also not significant.

Wilkins et al. (30) determined the unbound concentrations of acyclovir in horses after i.v. and p.o. administration. However, since the plasma protein binding of acyclovir is low, our results obtained for the total plasma concentration can be compared with those obtained by Wilkins et al. (30), as the same dosage was used and as both sets of data were analyzed according to a two-compartment PK model. The peak concentrations after i.v. acyclovir administration were similar, i.e., 10.7 g/ml in this study versus 10.4 g/ml in the study of Wilkins et al. (30). The mean AUC in this study (18.0 g·h/ml) was also similar to the one reported by Wilkins et al. (30) (19.2 μg·h/ml), together with other PK parameters, such as total body clearance and the apparent volume of distribution at steady state. The $t_{1/2}$ of elimination and the mean residence time were somewhat shorter in our study, i.e., 5.05 h and 5.38 h, respectively, in our study versus 9.60 h and 7.09 h, respectively, in the study of Wilkins et al. (30). However, as the $t_{1/2}$ of elimination was expressed as a harmonic mean in both studies and as large variations were noted among the various horses in our study, we can presume that these differences are not significant. Wilkins and colleagues (30) were unable to calculate the PK parameters after p.o. administration of acyclovir, as the plasma concentrations were below the lower limit of detection in all animals (LOQ, 156 ng/ml). Also in our study, a low $C_{\text{max}}$ of 0.33 ± 0.14 μg/ml was achieved 1 h after administration. At 5 h after administration, the plasma concentrations were below the LOQ of 50 ng/ml.
Our results obtained for the unbound concentration can be compared with the results of the study of Bentz et al. (5), as both sets of data were analyzed according to a three-compartment pharmacokinetic model, although Bentz et al. determined the total concentration. The $C_{\text{max}}$ after i.v. administration with the same dosage was much higher (46.2 µg/ml) in the study of Bentz et al. (5), which can be explained by an infusion duration of 15 min instead of the 1 h used in this study. We preferred a 1-h constant-rate infusion, based on the recommendations for parenteral administration in humans. The mean AUC after i.v. administration in our study (24.6 µg · h/ml) was lower than the one reported by Bentz et al. (5) (37.3 µg · h/ml); as a consequence, the total body clearance was higher (0.41 liter/h · kg in our study versus 0.30 liter/h · kg in the study of Bentz et al. [5]). Moreover, the apparent volume of distribution at steady state in this study (9.81 liters/kg) was also higher than that in the study of Bentz et al. (5) (3.54 liters/kg), which explains the similar $t_{1/2}$ of the terminal elimination phase. After the intragastric administration of acyclovir at a single dose of 20 mg/kg, Bentz et al. (5) reported an $F$ of 2.80%, while we found a value of 7.52%. This can be explained by the lower LOQ in our study compared to the one in the study of Bentz et al. (5) (40 ng/ml). This low $F$ of acyclovir limits the therapeutic use of acyclovir in infected horses. The barrier to absorption may be partly attributed to the limited solubility characteristics of the drug. As predicted by the pK<sub>a</sub> values of acyclovir (2.27 and 9.25), solubility is minimal between pH 2.3 and 9.2 (1.0 mg/ml). In the stomach, the solubility would then depend on the acidity in the stomach. Next to solubility problems, the oral absorption of acyclovir appears to be attributed to passive nonionic diffusion in humans (25) as well as in the rat (18). This diffusion is limited due to the log $P$ value of acyclovir, which is negative (log $P = -1.59$). In this study, the horses were fasted, which does not mimic the likely clinical application. This may have some impact on the absorption of the drug. However, in humans, it does not seem that food influences the absorption of acyclovir (9).

The oral prodrug valacyclovir is associated with a higher $F$ in humans (54% for valacyclovir versus 12 to 20% for acyclovir) (20). Also in our study, there was an eightfold increase in $F$, i.e., from 3.13% for acyclovir to 26.1% for valacyclovir. The addition of the valine moiety to acyclovir results in a substrate for active transport mechanisms in the intestinal tract and, thus, in a higher $F$ (16). After uptake, valacyclovir undergoes rapid and extensive first-pass intestinal and/or hepatic metabolism (hydrolysis) to yield acyclovir and t-valine (22). An additional study with two horses demonstrated that food had no influence on the absorption of valacyclovir in horses. There was no difference in the plasma peak concentrations between nonfasted horses, horses that were fasted 12 h prior to administration to 4 h after administration, and horses that were fasted during the entire sampling period (data not shown).

A recent study of the susceptibilities of six isolates of EHV-1 to acyclovir in vitro demonstrated 50% reduction of plaque formation (EC<sub>50</sub> value) at acyclovir concentrations of 1.7 to 3.0 µg/ml, depending on the isolate (12). After i.v. infusion over 1 h of 10 mg/kg of acyclovir and after single p.o. administration of 20 mg/kg valacyclovir, plasma levels could be maintained above these EC<sub>50</sub> values for 1.5 to 2 h. This demonstrates that the i.v. infusion of acyclovir has limited clinical applicability in cases of EHV-1 infection in horses, as this treatment should be useful only as a continuous infusion to exceed concentrations greater than 1.7 to 3.0 µg/ml for the entire treatment period. The higher $F$ of valacyclovir compared to that of acyclovir in horses results in a 10-fold higher $C_{\text{max}}$. As acyclovir has a relatively long $t_{1/2}$ of elimination in horses, it is likely that the accumulation of the drug could occur after repeated dosing.

Although the use of PK/PD approaches is not new to the pharmacology requirements for registration procedures, PK/PD approaches for antiviral therapy in veterinary medicine have not been applied, to our knowledge. On the basis of the knowledge that exists in antibiotic therapy, we can design an appropriate dosage regimen by combining PK and PD information. Several integrated PK/PD predictors of clinical and bacteriological outcomes have been proposed for antibiotic therapy. The three most used and useful are (i) the AUC/MIC ratio, an index used for, e.g., quinolones; (ii) the $C_{\text{max}}$/MIC ratio, an index selected for concentration-dependent antibiotics, such as aminoglycosides; and (iii) the time during which plasma concentrations exceed the MIC, expressed as a percentage of the dosage interval, an index selected for the so-called time-dependent antibiotics, such as β-lactams and macrolides. On the basis of the mode of action and the dosing schedules for acyclovir for the treatment of human genital herpes or herpes zoster infections, where the dose is given five times per day over 7 to 10 days, the time during which plasma concentrations exceed the EC<sub>50</sub> value seems to be the most suitable PK/PD predictor for antiviral treatment. Tod et al. (26) suggested that maximal efficacy is reached when the length of time that the acyclovir concentrations remain above the EC<sub>50</sub> is greater than 12 h in each 24-h period of treatment. The PKs of acyclovir are linear in humans at a dose between 0.5 mg/kg and 1 mg/kg (7) and in the dog at a dose between 5 mg/kg and 20 mg/kg (15). If it is assumed that the kinetics are also linear in horses, the nonparametric superposition tool of the WinNonLin software can be used. By using this tool and adjusting the average concentration-time data for the p.o. administration of valacyclovir, a dosage schedule was designed that makes it possible to obtain plasma concentrations above the EC<sub>50</sub> value of EHV-1 during the majority of the dosage interval. By dosing a horse with 40 mg of valacyclovir/kg body weight every 8 h, plasma concentrations are predicted to be higher than 1.7 µg/ml during the entire treatment interval and higher than 3.0 µg/ml during 30% of the treatment interval. Although this dosing is labor-intensive, this could be an achievable treatment for horses infected with EHV-1. Further studies are warranted to determine if repeated p.o. administration of the drug indeed results in plasma concentrations at the target concentration of 1.7 to 3.0 µg/ml during the majority of the treatment interval and if acyclovir is able to diffuse into the various tissues.

In conclusion, valacyclovir may be an attractive and valuable candidate for the treatment of EHV-1 infections in horses. This study also demonstrates that acyclovir administered p.o. at a dose of 20 mg/kg does not result in plasma concentrations that exceed the EC<sub>50</sub> value of EHV-1. Therefore, p.o. acyclovir is not useful for treatment.
ACKNOWLEDGMENTS

We thank Marc Vandenhauwe from GlaxoSmithKline for the kind gift of Zovirax IV, Zovirax 200, and Zelitrex 500. We also thank N. Desmet for the LC-MS/MS analysis of the samples.

REFERENCES