Pharmacokinetics of acyclovir after intravenous infusion of acyclovir and after oral administration of acyclovir and its prodrug valacyclovir in healthy adult horses.

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Abstract

The purpose of this study was twofold. The first aim was to evaluate the oral bioavailability and pharmacokinetics (PK) of acyclovir in horses after intravenous administration and after oral administration of acyclovir and its prodrug valacyclovir. Secondly, we aimed to combine these PK data with pharmacodynamic (PD) information, i.e. EC₅₀-values from in vitro studies, to design an optimal dosage schedule. Three treatments were performed in healthy adult horses: 10 mg of acyclovir/kg body weight delivered as an IV infusion over 1 hour, 20 mg of acyclovir/kg and 20 mg of valacyclovir/kg as tablets by nasogastric intubation. Total plasma concentrations were measured using a HPLC-method combined with fluorescence detection; while unbound plasma concentrations were determined using LC-MS/MS. Peak concentration for IV acyclovir was approximately 10 µg/ml for both total and unbound plasma concentration. 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 Cₘₐₓ and poor bioavailability. A 10-times higher Cₘₐₓ and an 8-times higher bioavailability were achieved with oral administration of valacyclovir. IV administration of 10 mg/kg acyclovir and OR 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 prophylactic and/or therapeutic treatment of horses infected with EHV-1. Results from PK/PD modelling showed that a dosage of 40 mg/kg valacyclovir, administered 3 times daily, would be sufficient to reach plasma concentrations above the EC₅₀-values.

Keywords: pharmacokinetics, acyclovir, valacyclovir, horses, herpesvirus
1 Introduction

Equine herpesvirus type 1 (EHV-1), a member of the 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 with 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 susceptibility of 6 isolates of EHV-1 to several antivirals, i.e. acyclovir, ganciclovir, cidofovir, adefovir, 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) and foscarnet (12). Based on the EC$_{50}$-value for inhibition of plaque formation, ganciclovir emerged as the most potent compound against all 6 isolates. Acyclovir was approximately 10-fold less effective (EC$_{50}$-value of 1.7 – 3.0 µg/ml). However, due to reported hematotoxicity in humans (2), the specific indications for cytomegalovirus infections in humans and the high cost price of ganciclovir, acyclovir seems a more attractive candidate for antiviral therapy against EHV-1 infection in horses.

Several recent reports describe the use of the antiviral drug acyclovir for the treatment of neurological disorders (14, 27, 29), as for the management of the neonatal form of EHV-1 infection (19). The benefit of these treatments is difficult to evaluate since there were no untreated control animals included. The dosages used in these studies were extrapolated from dosages recommended in human medicine and were not based on the pharmacokinetic properties of acyclovir in the horse.
Recently, the pharmacokinetics of acyclovir after intravenous (IV) and oral administration have been determined in horses (5, 30). IV administration of acyclovir achieved plasma concentrations within the sensitivity range described for EHV-1. For practical application during an outbreak, an oral form would be preferred rather than repeated IV infusion. However, orally administered acyclovir resulted in a 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 men. In humans, a 3-5 times higher bioavailability has been found for valacyclovir compared to acyclovir, i.e. 54% for valacyclovir versus 12-20% for acyclovir (20).

The first aim of the present study was to evaluate the oral bioavailability and pharmacokinetics of total and unbound acyclovir after intravenous administration of acyclovir and after oral administration of acyclovir and its prodrug valacyclovir. The unbound concentration of acyclovir particularly may be of interest as this is the concentration that can reach the biophase and has an effect on the viral replication. The second aim was to combine PK and PD information to design an optimal dosage regimen, so that plasma concentrations exceed the \( EC_{50} \)-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 exceed the \( EC_{50} \)-value of the virus determined \textit{in vitro} (8, 26).

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

2 Materials and methods
2.1 Animals

Six healthy adult horses (3 males, 3 females), aged 3.2 ± 1.3 years (mean ± SD) and weighing 446 ± 54 kg were studied using a three-way cross-over design with a 1-week wash-out period between treatments. Horses were weighed the day before each treatment. They were housed in individual stables, where they had continuous access to hay and water. A single-lumen IV catheter for blood sampling (Cavafix® Certo®), B Braun, Diegem, Belgium) was aseptically placed in one jugular vein prior to the start of the study. For the IV study, a short IV catheter (Intraflon 2, Vygon, Brussels, Belgium) was placed in the contralateral jugular vein during 1 hour for acyclovir administration. For the PO treatments, horses were fasted 12 hours before and 4 hours after administration. All procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University (EC 2005/44).

2.2 Drug administration and blood sampling

Treatments consisted of IV acyclovir at a dosage of 10 mg/kg and acyclovir or valacyclovir at 20 mg/kg intragastrically (PO). All the products were kindly donated by GlaxoSmithKline (Genval, Belgium). The calculated dose of injectable acyclovir (Zovirax™ I.V., Acyclovir for Injection 250 mg) was added to isotonic saline to a total amount of 300 ml and was given through the short IV catheter by constant rate infusion using a fluid pump (IVAC Star-Flow 580, IVAC Corporation, San Diego, CA, USA) over 1 hour. The PO doses were prepared identically for all horses by suspending crushed acyclovir tablets (Zovirax™ 200, Acyclovir Tablets 200 mg) or valacyclovir tablets (Zelitrex™ 500, Valaciclovir Tablets 500 mg) in 100 ml of tap water, followed by administration through a nasogastric tube. Nasogastric tubes were flushed with at least 200 ml of water immediately after administration.
Baseline (time 0) samples were collected just before drug administration. During IV administration of acyclovir, plasma samples were collected at 20, 40 and 60 minutes (end of infusion). Additional samples were obtained at 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 16, 23, 30 and 48 hours after the start of the infusion. For the PO study, plasma samples were collected at 20, 40 and 60 minutes, and at 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 16, 23 and 30 hours post administration. Samples were collected into heparinized blood collection tubes (Venoject, Terumo, Leuven, Belgium) and plasma was immediately harvested by centrifugation at 400 \( g \) for 10 minutes. Plasma was frozen at -70 °C until assayed.

Blood samples were also collected once daily, on the day before administration, the day of administration and the day after administration to evaluate the kidney function by measuring urea and creatinine values.

### 2.3 Sample analysis

First, a HPLC-fluorescence method was developed to measure the total concentration of acyclovir in plasma (limit of quantification (LOQ) = 50 ng/ml). With this method, we were able to determine the pharmacokinetics of acyclovir in horse plasma. However, at a later stage a more sensitive method was also developed to be able to quantify very low concentrations, not only in plasma, but also in nasal fluid, white blood cells and cerebrospinal fluid. These matrices play an important role in the pathogenesis of the virus. If acyclovir is able to penetrate into these body compartments, it may have an influence on the replication of the virus. Therefore, an LC-MS/MS method was developed which was 10-times more sensitive (LOQ = 5 ng/ml). As only the unbound concentration is able to diffuse into the tissues, we focussed on this fraction with the LC-MS/MS method.
2.3.1 Total plasma concentration

The quantification of acyclovir was based on the use of an internal standard (IS), ganciclovir, which was added to plasma just before analysis. To 1.0 ml of plasma, 50 µl of the IS solution (1 µg/ml for concentrations from 50 – 2500 ng/ml and 2 µg/ml for concentrations from 5000 – 20,000 ng/ml) were added. The samples were vortexed for 30 seconds and then subsequently deproteinized by the addition of 100 µl of pentafluoropropionic acid (PFPA).

After vortex mixing for 1 minute and centrifugation for 10 minutes at 10,000 g, the supernatant was transferred to another tube. Next, 5 ml of dichloromethane-isopropyl alcohol (50/50, v/v) were added. After vortex mixing for 30 seconds, the samples were allowed to extract for 10 minutes by gentle rolling. Then, samples were centrifuged for 10 minutes at 900 g 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 A and a volume of 100 µl was injected into the HPLC-fluorescence system.

The HPLC system consisted of a quaternary gradient pump P1000XR, an autosampler AS3000 with cooling device at 12 °C and a fluorescence detector type Jasco FP-920, all from Thermo Separation Products (San Jose, CA, USA). Chromatographic conditions were achieved using an Inertsil 5 ODS-3 column (250 x 4.6 mm I.D., kept at room temperature) in combination with a guard column (SS 10 x 2 mm) from Varian (Middelburg, The Netherlands). The mobile phase consisted of heptane sulfonic acid buffer (A) (0.05 M heptane sulfonic acid sodium salt in water and pH adjusted with phosphoric acid 85 wt. % solution to a pH of 2.5) and methanol (B), 90% A / 10% B. Gradient elution was performed at a flow rate of 1 ml/min (0-22 min 90% A – 10% B, 22-30 min 50% A – 50% B and 30-40 min 90% A – 10% B). The eluate was monitored with fluorescence detection at λ_{ex} = 270 nm and λ_{em} = 360 nm.
2.3.2 *Unbound plasma concentration*

Two hundred µl of plasma and 50 µl of the IS solution of 0.1 µg/ml were added to a Microcon® YM-30 centrifugal filter device (30,000 MWCO; molecular weight cut-off, Millipore, Brussels, Belgium). The samples were vortexed for 30 seconds and then centrifuged for 15 minutes at 10,000 g. 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, USA). Chromatography was performed using a Nucleodur C18 Pyramid column (125 x 2 mm I.D., 3 µm), in combination with a precolumn filter (CC 8/3 Nucleodur C18 Pyramid, 3 µm, Machery-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 (0-5.5 min 95% A – 5% B, 5.5-10 min 30% A – 70% B and 10-19 min 95% A – 5% B).

The mass spectrometer was a TSQ-Quantum triple quadrupole instrument from Thermo Electron Corporation, equipped with an electrospray ionization (ESI) 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 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, aux gas pressure 4 and capillary temperature 350 °C.

The instrument was operated in the selected reaction monitoring (SRM) mode, using the product ion mass-over-charge ratio \(m/z\) 152.2 for acyclovir and ganciclovir. The SRM
transitions at \( m/z \) [226->152.2] for acyclovir and at \( m/z \) [256->152.2] for ganciclovir were monitored from 2.5 to 6.5 minutes.

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

2.4 Plasma-protein binding study

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

\[
\text{bound drug (\%)} = \left( \frac{\text{Total Plasma Concentration} - \text{Unbound Plasma Concentration}}{\text{Total Plasma Concentration}} \right) \times 100 \%
\]

2.5 Pharmacokinetic analysis

Analysis of drug concentration-time curves, compartmental and non-compartmental pharmacokinetic modelling were performed using a computerized program (WinNonLin v5.01, Pharsight, Mountain View, CA, USA). The best fitting model was selected after visual inspection of the fitted curve, a smaller value for the 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 IV administration was best described by a 2-compartmental model, while the unbound plasma concentration after IV administration was best described by a 3-compartmental model. The corresponding equations during and after infusion were:

\[ C_t = \sum_{i=1}^{n} \frac{C_i}{\lambda_i * T} \left(1 - e^{-\lambda_i t}\right) \quad (t < T) \]

\[ C_t = \sum_{i=1}^{n} \frac{C_i}{\lambda_i * T} \left(e^{-\lambda_i t} - e^{-\lambda_i T}\right) \quad (t \geq T) \]

In these equations \( C_t \) is the plasma drug concentration at time \( t \), \( T \) is the infusion duration and \( C_i, \lambda_i \) are the coefficients and exponents of the equation, respectively (13).

The elimination half-life (T\(_{1/2}\)) was estimated from the relationship:

\[ T_{1/2} = \frac{\ln 0.5}{k_e} \]

where \( k_e \) represents the elimination rate constant, i.e. \( \beta \) and \( \gamma \) for the 2- and 3-compartmental model, respectively. The area under the curve (AUC\(_{t \rightarrow \infty}\)) was calculated using the linear trapezoidal method for AUC and adding the estimated terminal portion of the curve (\( C_i/k_e \)), where \( t \) is the last time of measurable plasma concentrations.

The data after the oral administrations were analyzed using non-compartmental methods (11). The absolute oral bioavailability (F) of acyclovir and valacyclovir was determined using the equation:

\[ F (%) = \frac{AUC(PO)}{AUC(IV)} \times \frac{Dose(IV)}{Dose(PO)} \times 100\% \]

To determine whether PK parameters differed for the total and unbound drug, statistical analysis was performed based on analysis of variance (ANOVA) using SPSS (SPSS Inc., Chicago, IL, USA).
2.6 PK/PD index and PK/PD breakpoint

The PK/PD index that was used is Time>EC$_{50}$, defined as the cumulative percentage of time over a 24 hour period that the drug concentration exceeds the EC$_{50}$ (4). This is expressed as a percentage of the dosage schedule, which is the PK/PD breakpoint and should be more than 50% (26).

3 Results

No adverse effects were observed in any of the 6 horses. The major reported side effect of parenteral administration of acyclovir in humans is renal toxicity. This is related to 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 function was monitored in all 6 horses. No significant changes in urea and creatinine levels were seen during this trial, compared to the baseline value of day-1 (P>0.05).

3.1 Total plasma concentration

The mean total plasma acyclovir concentration versus time curve after IV administration of acyclovir and after oral dosing of acyclovir and valacyclovir is shown in Figure 1. Table 1 presents the pharmacokinetic parameters after IV administration. Peak concentration (C$_{max}$) for IV acyclovir was 10.7 ± 2.79 µg/ml (mean ± SD). The harmonic mean half-life of the distribution phase was 0.23 h, while the harmonic mean half-life of the elimination phase was 5.05 h. The volume of distribution at steady state was 2.96 ± 2.09 l/kg.
After intragastric administration of acyclovir, oral bioavailability was only 3.13 % and plasma concentrations dropped below the LOQ of the HPLC-fluorescence method after 5 h.

Intragastric administration of valacyclovir was associated with a bioavailability 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 half-life of the elimination phase was 2.03 h (Table 2).

3.2 Unbound plasma concentration

The mean unbound plasma acyclovir concentration versus time curve after IV administration of acyclovir and after oral dosing of acyclovir and valacyclovir is shown in Figure 2. Table 1 presents the pharmacokinetic parameters after IV administration. Peak concentration (mean ± SD) for IV acyclovir was 10.5 ± 3.98 µg/ml. The harmonic mean half-life of the rapid-distribution phase, the slow-distribution phase and the elimination phase was 0.10 h, 1.23 h and 11.9 h, respectively. The volume of distribution at steady state was 9.81 ± 11.4 l/kg.

After intragastric administration of acyclovir, the oral bioavailability 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 oral bioavailability of 26.4 ± 7.11 % was obtained after 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 half-life of the elimination phase was 8.79 h (Table 2).

3.3 Plasma-protein binding
The mean value and standard deviation (n = 6) of the plasma protein binding capacity of acyclovir in pooled plasma from the 6 horses are presented in Figure 3a. A percentage binding between 10 and 20% was calculated. There was a tendency of a decreasing plasma protein binding with increasing concentrations. However, there was no significant difference between the various concentrations and the percentage binding (P>0.05). The mean and standard deviation of the plasma protein binding at 3 concentrations (0.5, 5 and 15 µg/ml) of the 6 individual horses is presented in Figure 3b. The differences between the horses were not significant (P>0.05).

3.4 PK/PD index

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

4 Discussion

The present study was conducted to evaluate the oral bioavailability and pharmacokinetics of total and unbound acyclovir after intravenous administration of acyclovir and after oral
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 men, oral administration of valacyclovir is associated with higher bioavailability than the parent drug. An 8-times higher bioavailability was noted for valacyclovir, resulting in plasma concentrations exceeding those which inhibit plaque formation of EHV-1 \textit{in vitro}. This strongly indicates clinical applicability for this drug in cases of EHV-1 infection in horses.

Similar plasma concentrations were reached for both total and unbound acyclovir. This can be explained by a rather low protein binding since free fractions vary between 0.8 and 0.9 in horses (Figure 3). However, with the LC-MS/MS method, 10-fold lower acyclovir concentrations could be detected compared to the HPLC-fluorescence method. Due to this improved detection sensitivity, a more prolonged elimination phase was observed and a 3-compartment model described best the data after IV 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 half-life of elimination seemed longer, i.e. 11.9 versus 5.05 h. However, no statistically significant differences could be detected (P>0.05). After intragastric administration of valacyclovir, maximum concentrations were already reached within 1 hour. Again, the mean half-life 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 concentration of acyclovir in horses after IV and oral administration. However, since the plasma protein binding of acyclovir is low, our results obtained for the total plasma concentration can be compared, as the same dosage was used and as both data were analyzed according to a 2-compartment pharmacokinetic model. Peak concentrations after intravenous acyclovir were similar, i.e. 10.7 µg/ml in this study.
versus 10.4 µg/ml. The mean AUC in this study (18.0 h*µg/ml) was also similar to the one reported in Wilkins’ study (19.2 h*µg/ml), together with other pharmacokinetic parameters, such as total body clearance and apparent volume of distribution at steady state. The half-life of elimination and mean residence time were somewhat shorter in our study, i.e. 5.05 h and 5.38 h versus 9.60 h and 7.09 h. However, as the half-life of elimination is 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 was unable to calculate the pharmacokinetic parameters after oral administration of acyclovir, as plasma concentrations were below the lower limit of detection in all animals (LOQ = 156 ng/ml). Also in our study, a low maximum concentration of 0.33 ± 0.14 µg/ml was achieved 1 hour after administration. Five hours after administration, 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 data were analyzed according to a 3-compartment pharmacokinetic model, although Bentz determined the total concentration. Peak concentration after intravenous administration with the same dosage was much higher in Bentz’ study (46.2 µg/ml), which can be explained by an infusion duration of 15 minutes instead of 1 hour. We preferred a 1 hour-constant rate infusion, based on the recommendations for parenteral administration in humans. The mean AUC after IV administration in our study (24.6 h*µg/ml) was lower than the one reported by Bentz (37.3 h*µg/ml); as a consequence the total body clearance was higher (0.41 l/h*kg versus 0.30 l/h*kg). Moreover, the apparent volume of distribution at steady state in this study (9.81 l/kg) was also higher compared to the study of Bentz (3.54 l/kg), which explains the similar half-life of the terminal elimination phase. After intragastric administration of acyclovir at a single dose of 20 mg/kg, Bentz reported an oral bioavailability of 2.80 %, while we found a value of
7.52%. This can be explained by a lower LOQ in our study, compared to the one in Bentz’ study (40 ng/ml). This low bioavailability of acyclovir limits the therapeutic use in infected horses. The barrier to absorption may be partly attributed to the limited solubility characteristics of the drug. As predicted by the pK\textsubscript{a}-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 non-ionic diffusion, as well in human (25) as in the rat (18). This diffusion is limited due to the logP-value of acyclovir which is negative (logP = -1.59). In this study, 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 bioavailability in humans (54% versus 12-20%) (20). Also in our study, there was an 8-fold increase in bioavailability, 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 bioavailability (16). After uptake, valacyclovir undergoes rapid and extensive first-pass intestinal and/or hepatic metabolism (hydrolysis) to yield acyclovir and L-valine (22). An additional study with 2 horses demonstrated that food had no influence on the absorption of valacyclovir in horses. There was no difference in plasma peak concentrations between non-fasted horses, horses that were fasted 12 hours prior to administration until 4 hours after administration and horses that were fasted during the entire sampling period (data notshown). A recent study of the susceptibility of 6 isolates of EHV-1 to acyclovir in vitro demonstrated 50% reduction of plaque formation (EC\textsubscript{50}-value) at an acyclovir concentration of 1.7 to 3.0 µg/ml, depending on the isolate (12). After IV infusion over 1 hour of 10 mg/kg of acyclovir and after single oral administration of 20 mg/kg valacyclovir, plasma levels could
be maintained above these EC\textsubscript{50}-values during 1.5 to 2 hours. This demonstrates that IV infusion of acyclovir has limited clinical applicability in cases of EHV-1 infection in horses as this treatment should only be useful as a continuous infusion to exceed concentrations greater than 1.7 to 3.0 µg/ml for the entire treatment period. The higher bioavailability of valacyclovir compared to acyclovir in horses results in a 10-fold higher peak concentration. As acyclovir has a relatively long half-life of elimination in horses, it is likely that accumulation of the drug could occur after repeated dosing.

Although 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. Based on 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) C\textsubscript{max}/MIC ratio, an index selected for concentration-dependent antibiotics such as aminoglycosides and (iii) T>MIC (the time during which plasma concentrations exceed 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. Based on the mode of action and on dosing schedules for acyclovir for treatment of human genital herpes or herpes zoster infections, where the dose is given 5 times/day over 7 to 10 days; the time during which plasma concentrations exceed the EC\textsubscript{50}-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\textsubscript{50} is greater than 12 h in each 24-h period of treatment. The pharmacokinetics of acyclovir are linear in men at a dose between 0.5 mg/kg and 1 mg/kg (7) and in dog at a dose between 5 mg/kg and 20 mg/kg (15). Assuming that the kinetics are also linear in horses, the non-parametric superposition tool of
WinNonLin can be used. Using this tool and the average concentration-time data of the oral administration of valacyclovir, a dosage schedule was designed that makes it possible to obtain plasma concentrations above the EC_{50}-value of EHV-1 during the majority of the dosage interval. By dosing a horse with 40 mg of valacyclovir/kg bodyweight, every 8 hours, 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 labour intensive, this could be an achievable treatment for horses infected with EHV-1. Further studies are warranted to determine if repeated oral 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 orally administered acyclovir at a dose of 20 mg/kg does not result in plasma concentrations exceeding the EC_{50}-value of EHV-1. Therefore, oral acyclovir is not useful for treatment.

Acknowledgment

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References


Fig. 1. Total plasma concentration versus time plot (mean + SD) of acyclovir in 6 horses during and following a 1-hour constant rate infusion of acyclovir at a dose of 10 mg/kg (—); after oral administration of acyclovir at 20 mg/kg (——) and after oral administration of valacyclovir at 20 mg/kg (······).
Table 1. Pharmacokinetic parameters (mean ± SD), derived from compartmental analysis, for total and unbound acyclovir administered as IV infusion (1h) to 6 horses at 10 mg/kg.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Total concentration</th>
<th>Unbound concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$</td>
<td>$\mu g/ml$</td>
<td>10.7 ± 2.79</td>
<td>10.5 ± 3.98</td>
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<tr>
<td>C</td>
<td>$\mu g/ml$</td>
<td>--</td>
<td>0.53 ± 0.35</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$1/h$</td>
<td>3.06 ± 0.52</td>
<td>6.83 ± 6.67</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$1/h$</td>
<td>0.14 ± 0.08</td>
<td>0.56 ± 0.24</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>$1/h$</td>
<td>--</td>
<td>0.06 ± 0.05</td>
</tr>
<tr>
<td>$T_{1/2\alpha}$</td>
<td>$h$</td>
<td>0.23*</td>
<td>0.10*</td>
</tr>
<tr>
<td>$T_{1/2\beta}$</td>
<td>$h$</td>
<td>5.05*</td>
<td>1.23*</td>
</tr>
<tr>
<td>$T_{1/2\gamma}$</td>
<td>$h$</td>
<td>--</td>
<td>11.9*</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>$1/h$</td>
<td>1.72 ± 0.56</td>
<td>1.66 ± 0.62</td>
</tr>
<tr>
<td>$k_{21}$</td>
<td>$1/h$</td>
<td>0.23 ± 0.11</td>
<td>0.88 ± 0.39</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>$1/h$</td>
<td>1.25 ± 0.34</td>
<td>2.39 ± 2.87</td>
</tr>
<tr>
<td>$k_{31}$</td>
<td>$1/h$</td>
<td>--</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>$k_{13}$</td>
<td>$1/h$</td>
<td>--</td>
<td>2.43 ± 3.12</td>
</tr>
<tr>
<td>$k_{13}$</td>
<td>$1/h$</td>
<td>--</td>
<td>2.43 ± 3.12</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>$1/h$</td>
<td>--</td>
<td>2.43 ± 3.12</td>
</tr>
<tr>
<td>$k_{13}$</td>
<td>$1/h$</td>
<td>--</td>
<td>2.43 ± 3.12</td>
</tr>
<tr>
<td>Cl</td>
<td>$l/h*kg$</td>
<td>0.59 ± 0.18</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>$V_1$</td>
<td>$l/kg$</td>
<td>0.36 ± 0.10</td>
<td>0.27 ± 0.09</td>
</tr>
<tr>
<td>$V_{ss}$</td>
<td>$l/kg$</td>
<td>2.96 ± 2.09</td>
<td>9.81 ± 11.4</td>
</tr>
<tr>
<td>AUC</td>
<td>$h*\mu g/ml$</td>
<td>18.0 ± 4.25</td>
<td>24.6 ± 2.42</td>
</tr>
<tr>
<td>MRT</td>
<td>$h$</td>
<td>5.38 ± 3.96</td>
<td>23.1 ± 25.5</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$: maximal plasma drug concentration; $A$, $B$: pre-exponential term for distribution and elimination phase (2-compartment) / $A$, $B$, $C$: pre-exponential term for rapid distribution, slow distribution and elimination phase (3-compartment); $\alpha$, $\beta$, $\gamma$: exponential term for distribution and elimination phase (2-compartment) / $\alpha$, $\beta$, $\gamma$: exponential term for rapid distribution, slow distribution and elimination phase (3-compartment); $T_{1/2\alpha}$, $T_{1/2\beta}$: distributional and elimination half-life (2-compartment) / $T_{1/2\alpha}$, $T_{1/2\beta}$, $T_{1/2\gamma}$: rapid distributional, slow distributional and elimination half-life (3-compartment); $k_{10}$, $k_{12}$, $k_{21}$: distribution and elimination microconstants for 2-compartment model / $k_{10}$, $k_{12}$, $k_{21}$, $k_{13}$, $k_{31}$: distribution and elimination microconstants for 3-compartment model; Cl: total body clearance; $V_1$: volume central compartment; $V_{ss}$: apparent volume of distribution at steady state; AUC: area under the plasma drug concentration-time curve; MRT: mean residence time.

* Harmonic mean
Table 2. Pharmacokinetic parameters (mean ± SD), derived from non-compartmental analysis, for total and unbound acyclovir determined after a single 20 mg/kg dose of acyclovir and valacyclovir administered orally to 6 horses.

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

* Harmonic mean
Fig. 2. Unbound plasma concentration versus time plot (mean ± SD) of acyclovir in 6 horses during and following a 1-hour constant rate infusion of acyclovir at a dose of 10 mg/kg (—●—); after oral administration of acyclovir at 20 mg/kg (—■—) and after oral administration of valacyclovir at 20 mg/kg (···▲···).
Fig. 3a. Mean plasma protein binding capacity (+ SD) of acyclovir in pooled plasma from 6 horses at a concentration of 0.5, 5 and 15 µg/ml.

Fig. 3b. Plasma protein binding capacity of acyclovir in 6 individual horses (A till F), presented as a mean (+ SD) of 3 concentrations (0.5, 5 and 15 µg/ml).
Fig. 4. Prediction of the plasma concentration versus time plot after oral administration of 40 mg/kg valacyclovir every 8 hours.