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Pharmacokinetics of ganciclovir and valganciclovir in the adult horse

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Abstract

Equine herpes myeloencephalopathy, resulting from equine herpes virus type 1 (EHV-1) infection, is associated with substantial morbidity and mortality in the horse. As compared to other antiviral drugs, such as acyclovir, ganciclovir has enhanced potency against EHV-1. This study investigated the pharmacokinetics of ganciclovir and its oral prodrug, valganciclovir, in six adult horses in a randomized cross-over design. Ganciclovir sodium was administered intravenously as a slow bolus at a dose of 2.5 mg/kg and valganciclovir was administered orally at a dose of 1800 mg per horse. Intravenously administered ganciclovir disposition was best described by a three compartment model with a prolonged terminal half-life of 72 ± 9 hours. Following the oral administration of valganciclovir, the mean observed maximum serum ganciclovir concentration was 0.58 ± 0.37 $\mu\text{g/mL}$, and bioavailability of ganciclovir from oral valganciclovir was $41 \pm 20\%$. Superposition predicted that oral dosing of 1800 mg valganciclovir two times daily would fail to produce and maintain effective plasma concentrations of ganciclovir. However, superposition suggested that i.v. administration of ganciclovir at 2.5 mg/kg every 8 hours for 24 hours followed by maintenance dosing of 2.5 mg/kg every 12 hours would maintain effective ganciclovir serum concentrations in most horses throughout the dosing interval.

Keywords

Herpesvirus; horses; ganciclovir; pharmacokinetics; antiviral

INTRODUCTION

Equine herpes virus type-1 (EHV-1) is responsible for multiple disease syndromes in the horse. The virus is commonly associated with respiratory disease in young horses, abortion in mares, and neonatal foal death (Kydd et al., 2006; Lunn et al., 2009). However,

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neurologic disease has long been recognized as a particularly devastating manifestation of EHV-1 infection (Saxegaard, 1966). Initially, EHV-1 infects the respiratory epithelium, followed by spread to respiratory lymph nodes. A resulting leukocyte-associated viremia facilitates spread of the virus to other tissues such as the uterus or central nervous system (CNS) (Edington et al., 1986). Neurologic signs are believed to result from endothelial damage within the CNS, causing ischemic necrosis of the spinal cord. Research into different EHV-1 strains has revealed that a single point mutation within the DNA polymerase is strongly associated with the development of neurologic disease (Nugent et al., 2006; Goodman et al., 2007; Perkins et al., 2009).

Recent outbreaks of equine herpes myeloencephalopathy (EHM) have raised the concern that neurologic disease associated with EHV-1 may be an emerging threat (USDA, APHIS, 2007), and an effective treatment for EHM has yet to be substantiated (Lunn et al., 2009). Previous studies have investigated acyclovir and its prodrug valacyclovir for their use against EHV-1 (Wilkins et al., 2003; Wilkins et al., 2005; Bentz et al., 2006; Garre et al., 2007a; Maxwell et al., 2008a; Garre et al., 2009a; Garre et al., 2009b). Bioavailability of oral acyclovir has been shown to be poor (~4 %) in the horse, making it unappealing for therapeutic use (Bentz et al., 2006). However, bioavailability of acyclovir from its prodrug, valacyclovir, is considerably higher at 26–60%, making oral valacyclovir a more attractive therapeutic option than oral acyclovir (Maxwell et al., 2008a; Garre et al., 2009b). Although results using different models of EHV-1 infection are conflicting, valacyclovir administration has shown promise in reducing viral shedding, pyrexia, clinical disease, and neurologic severity in experimentally infected horses when given prophylactically or early in the disease course (Maxwell et al., 2008b; Garre et al., 2009b). However, as an EHV-1 outbreak may not be recognized until some horses are in imminent danger of developing neurological signs, more potent and predictable antiviral drugs may be necessary later in the course of the disease. In a study comparing the *in vitro* efficacy of several antiviral drugs against EHV-1, ganciclovir was demonstrated to be the most potent inhibitor of the virus (Garre et al., 2007b). This potency may translate into more efficacious therapy for EHV-1 as compared to less potent drugs, such as acyclovir. Similar to formulations of acyclovir, ganciclovir is also available for use in humans as both an injectable formulation and as a valine ester prodrug, valganciclovir, which circumvents the poor oral bioavailability of ganciclovir itself. The purpose of this study was to investigate the pharmacokinetics of intravenous ganciclovir and of its oral prodrug, valganciclovir and to design safe and effective dosing regimens for each formulation for further testing of multiple dose pharmacokinetics of these drugs in horses.

MATERIALS AND METHODS

Study design

All procedures were approved by the Institutional Animal Care and Use Committee at Oklahoma State University. Six lightbreed horses, including three mares and three geldings (557 ± 59 kg body weight; 6 ± 4 years of age) were randomly assigned to one of two treatment groups in a complete cross-over design. Horses were treated either with injectable ganciclovir (Cytovene™, Roche Laboratories Inc., Nutley, NJ USA) at 2.5 mg/kg

administered intravenously (i.v.) or valganciclovir (Valcyte™, Laboratories Inc., Nutley, NJ USA) at 1800 mg per horse administered orally. A two week washout period was allowed between treatments. Commercial ganciclovir sodium in 500 mg vials was reconstituted with 10 mL of sterile water, resulting in a final ganciclovir concentration of 50 mg/mL. Ganciclovir sodium was administered as a slow bolus over approximately 2 minutes through a jugular vein catheter while horses were observed for adverse effects. A dose of approximately 2.5 mg/kg of valganciclovir for a 450 kg horse was calculated and administered using 450 mg tablets rounded to the nearest whole tablet, such that each horse received 1800 mg valganciclovir. Adjusting for the molecular weight of valganciclovir (390.83) as compared to that of ganciclovir (255.23), horses administered valganciclovir received 2.15 ± 0.21 mg/kg of ganciclovir orally. In order to minimize exposure of personnel to valganciclovir, the tablets were not split or crushed. Because valganciclovir is stable in acidic aqueous solutions but not at a basic pH (Stefanidis & Brandl, 2005), it was first dissolved in lemon juice, then mixed with syrup and flour to produce a paste, followed immediately by oral administration with a syringe.

Baseline (time 0) plasma samples were collected from all horses prior to drug administration. After i.v. administration of ganciclovir, blood samples were collected from a separate i.v. catheter in the opposite jugular vein at 3, 5, 10, 15, 20, 30, 45 minutes and 1, 1.5, 2, 3, 6, and 8 hours. Further sampling was performed by jugular venipuncture at 12, 24, 48, 72, 120, and 168 hours after administration. Following oral administration of valganciclovir, blood samples were obtained at 10, 20, 30, 45 minutes, and 1, 1.5, 2, 3, 6, and 8 hours through an intravenous jugular catheter. Further sampling was performed by jugular venipuncture at 12, 24, 48, 72, 120, 168 hours. All samples were collected into heparinized blood collection tubes (Monoject,™ Tyco, Mansfield, MA USA) and placed immediately in ice. Samples were then centrifuged and plasma was separated and stored at -40°C until assayed. Long-term stability of ganciclovir in samples stored at 20°C has been previously reported and was not repeated in the present study (Chu et al., 1999).

HPLC assay

Plasma concentrations of ganciclovir were determined using high performance liquid chromatography (HPLC) with fluorescence detection. The HPLC system consisted of a ProStar™ 210 pump, 410 autosampler and 363 fluorescence detector (excitation: 260 nm, emission: 375 nm; Varian Corp., Walnut Creek, CA, USA). A reversed-phase, polar-embedded column and guard column (Symmetry Shield™ RP18, 5 μm , 250×4.6 mm; Waters Corp, Milford, MA, USA) were utilized at 30°C for analyte separation. Mobile phase components were 25 mM potassium phosphate and 5 mM octanesulphonic acid adjusted to a pH of 2.1 with phosphoric acid (mobile phase A), and 7.5 mM potassium phosphate and 5 mM octanesulphonic acid adjusted to a pH of 2.1 in 35% acetonitrile and 35% methanol (mobile phase B). Plasma calibrants were prepared at concentrations of 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.25, and 3.0 $\mu\text{g/mL}$ using ganciclovir (Sigma, St. Louis, MO USA) stock solutions in water and heparinized plasma from unmedicated horses. Calibrants and quality control samples were prepared by adding 975 μL of unmedicated equine heparinized plasma to 25 μL of the appropriate calibrant or quality control solution, followed by vortex mixing. One milliliter of experimental equine plasma was used for analysis. One milliliter of 2%

phosphoric acid containing the internal standard, penciclovir (LKT Lab., Inc., St. Paul, MN, USA), at a concentration of 0.05 µg/mL was added to each plasma sample and vortex mixed. Three milliliter MCX Oasis™ solid phase extraction columns (Waters Corp., Milford, MA USA) were conditioned with one mL of methanol followed by one mL of 2% phosphoric acid. The acidified plasma samples were loaded onto the columns, which were washed with 1 mL of 2 % formic acid followed by washing with 3 mL of 1:1 methanol:acetonitrile. Samples were eluted using 1 mL of 5% ammonium hydroxide in methanol. Following elution, samples were dried at 55° C under nitrogen gas. The residue was dissolved in 300 µL of mobile phase A and 50 µL were injected onto the HPLC column. Initial conditions were 96% of mobile phase A and 4% of mobile phase B until 1 minute, increasing to 100% of solution B from 1 to 12 minutes, and then returned to 4% solution B for re-equilibration, all at a flow rate of one mL/min. The intraday accuracy and coefficient of variation (CV) for ganciclovir quality control samples at 0.04 µg/mL were 99.6% and 1.5% and at 2 µg/mL were 102.6% and 0.8%, respectively. The interday accuracy and CV for ganciclovir quality control samples at 0.04 µg/mL were 100.2% and 0.8% and at 2 µg/mL were 104% and 1.9%, respectively. The limit of quantitation (LOQ) for the assay was 0.005 µg/mL and was defined by the lowest concentration at which accuracy and precision were within 20% of expected values. The limit of detection was not determined.

Pharmacokinetic analysis

Ganciclovir concentrations were analyzed compartmentally using WinNonlin Professional™ (Pharsight Corp., Mountain View, CA USA). Intravenous data were fit to the following equation:

$$C = \sum_{i=1}^n A_i \cdot e^{-\lambda_i \cdot t}$$

A lag time was included for the oral data to account for the time required to convert valganciclovir to ganciclovir (Wiltshire et al., 2005; Zhao et al., 2009). The most appropriate model for each route of administration was determined by using Akaike's information criterion and inspection of ganciclovir concentration versus time plots with residuals. The area under the plasma ganciclovir concentration versus time curve until infinity (AUC_{total}) for ganciclovir after i.v. administration and oral administration of valganciclovir was calculated noncompartmentally from the sum of trapezoids.

The bioavailability (F) of ganciclovir after oral administration of valganciclovir was estimated using the following equation:

$$F\% = \frac{AUC_{PO}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{PO}} \times 100$$

For the calculation of bioavailability, the dose of ganciclovir administered as valganciclovir was determined by dividing the molecular weight of the active drug (255.23) by that of its prodrug (390.83) and multiplying the resulting ratio (0.65) by the total administered dose of valganciclovir. Superposition was applied to the mean concentration versus time data

following i.v. ganciclovir and oral valganciclovir administration to predict ganciclovir concentrations that would be associated with multiple dosing regimens (Thron, 1974; Wang and Ouyang, 1998; Pollina et al. 2011). The target range of 0.1 – 0.4 µg/mL for ganciclovir trough concentrations was chosen based on previously published inhibitory (IC₅₀) values for ganciclovir susceptibilities against multiple EHV-1 isolates (Garre et al., 2007b). Dosing regimens were designed to achieve target plasma concentrations within 24 hours of beginning antiviral therapy, because maximal drug utility requires that effective drug concentrations be rapidly attained during an EHV-1 outbreak. In addition, the predicted multiple dose regimen was designed to maintain ganciclovir concentration within the target range for the majority of the dosing interval, since antiherpetic drug efficacy depends on maintaining concentrations above the IC₅₀ for at least one-half of the dosing interval (Tod et al., 2001).

RESULTS

Intravenous administration of ganciclovir as a slow bolus over approximately two minutes was well tolerated by all horses. Following the i.v. administration of a single dose of ganciclovir sodium, plasma ganciclovir concentrations remained above the assay's LOQ throughout the sampling interval of seven days. Data were best described by a three-compartment model, demonstrating a rapid decline phase followed by a slower distribution phase and an extended elimination phase (figure 1). The mean ± SD estimated elimination half-life was prolonged but consistent among horses at 72 ± 9 hr, whereas the total body clearance was similarly consistent at 3.6 ± 0.7 mL·min⁻¹·kg⁻¹. The AUC_{total} was 12 ± 2.0 µg·hr/mL, and the terminal, elimination phase comprised the majority (67 ± 8 %) of the total drug exposure (table 1).

Following the administration of a single oral dose of valganciclovir, plasma ganciclovir concentrations remained above the assay's LOQ through the third day of sampling in all six horses, but was only quantifiable in 2/6 horses by five days after administration. Ganciclovir was rapidly detectable in plasma after oral valganciclovir administration, but a lag period was required to accurately fit the data. The oral data were best described by a two-compartment model, due to distinct distribution and elimination phases (figure 2). Oral absorption was rapid, with an absorption half-life of 0.66 ± 0.26 hr. Maximal plasma ganciclovir concentrations of 0.58 ± 0.37 µg/mL were achieved at 1.3 ± 0.3 hr. The bioavailability of ganciclovir from valganciclovir was moderate and variable among horses, at 41 ± 20% (table 2).

Superposition suggested that administration of an initial loading regimen of 2.5 mg/kg of ganciclovir i.v. every 8 hours for 24 hours, followed by a maintenance regimen of 2.5 mg/kg every 12 hours would maintain ganciclovir plasma levels in the targeted zone of 0.1 – 0.4 µg/mL (figure 3). Alternatively, administration of a single loading dose of 5 mg/kg ganciclovir followed by 2.5 mg/kg every 12 hours was also predicted to maintain plasma levels at or above target concentrations. Superposition of the oral valganciclovir data suggested that administering 1800 mg valganciclovir every 12 hours would not result in plasma concentrations of ganciclovir reaching target trough concentrations within 24 hours

of beginning drug administration. Instead, administration of 3600 mg every 12 hours would be required to produce the targeted trough concentrations (figure 4).

DISCUSSION

Ganciclovir pharmacokinetics following i.v. administration were best described by a three-compartment model with an elimination phase half-life of 72 ± 9 hours. This prolonged elimination half-life of ganciclovir in horses was in contrast to that reported in other species, where average elimination half-lives were five hours or less (Serabe et al. 1999; Winston et al., 2006). Although the disposition of ganciclovir in horses differed considerably from that of other mammalian species, it was consistent with the pharmacokinetics of the related nucleoside analogs acyclovir and penciclovir in horses, where the elimination half-life was similarly prolonged (Garre et al., 2007a; Maxwell et al., 2008a; Tsujimura et al., 2010). Thus, it appears that horses may handle the nucleoside analogs in a fundamentally different manner than do most other species, with a prolonged terminal phase that has been posited to represent a deep compartment that slowly returns drug to circulation (Maxwell et al., 2008a). The elimination half-life of ganciclovir after oral administration of valganciclovir was also prolonged at 43 ± 20 hours, respectively. The somewhat shorter elimination half-life associated with valganciclovir administration most likely reflects the shorter period of time that ganciclovir was quantifiable after oral dosing, due to lower systemic ganciclovir exposure as compared to i.v. dosing. Pharmacokinetic studies of antiviral drugs such as acyclovir, valacyclovir, and famciclovir have consistently reported prolonged elimination half-lives in the horse (Garre et al., 2007a; Maxwell et al., 2008a; Tsujimura et al., 2010). This is in contrast to studies examining the pharmacokinetics of nucleoside analogs in most other species, where the elimination half-lives are considerably shorter. For example, the elimination half-life of ganciclovir in humans is approximately 2.5 hours (Krasny et al., 1981; Fletcher et al., 1986). The prolonged elimination rate of ganciclovir is probably not due to slow drug clearance, as the clearance rate of 3.6 ± 0.7 mL/min·kg was similar to that reported for acyclovir and to published values for glomerular filtration rate in the horse (Maxwell et al., 2008a; Wilson et al., 2009). This is consistent with reports in humans indicating ganciclovir is renally eliminated through glomerular filtration (Jacobson et al., 1987). The prolonged elimination half-life of antiviral drugs in the horse has instead been attributed to the presence of a deep compartment responsible for drug sequestration and slow release (Maxwell et al., 2008a).

Given the slow elimination rate of ganciclovir and the large contribution of the elimination phase to the AUC_{total} , it is likely that the accumulation of ganciclovir over multiple doses will be substantial in horses, in contrast to multi-dose kinetics reported in other species (Acosta et al., 2007). In addition, it is possible that linear kinetics will not be observed at extrapolated doses, since the current study only tested a single oral dose and a single intravenous dose rate. Therefore, further studies utilizing a multiple dose study design are warranted before clinical recommendations for multiple dosing regimens can be confidently made.

Ganciclovir was detected in plasma rapidly after oral administration of valganciclovir and maximal concentrations of ganciclovir were reached at 1.3 ± 0.3 hours after administration.

As additional support for the rapid absorption of ganciclovir following valganciclovir administration, the calculated absorption half-life ($t_{1/2(\text{abs})}$) was less than 1 hour in all horses. Zhao, Baudouin et al. (2009) compared different basic models of valganciclovir pharmacokinetics in humans and by evaluating residual variability determined that a two-compartment model with lag time and first-order absorption and elimination optimized valganciclovir modeling. Consistent with the studies of valganciclovir kinetics in humans and valacyclovir administration in horses, a lag time was necessary to account for the time required for conversion of valganciclovir to ganciclovir (Wiltshire et al., 2005; Maxwell et al., 2008a; Zhao et al., 2009).

Following the oral administration of 1800 mg of valganciclovir, bioavailability (F) was determined to be $41 \pm 20\%$ showing substantial variability between horses. It should be noted that the horses utilized in this study were not fasted, and that in humans, bioavailability was improved when valganciclovir was administered with a meal (Acosta et al., 2007). The bioavailability reported here reflects the molecular weight of ganciclovir (255.23) as compared to that of valganciclovir (390.83). Therefore, the bioavailability was determined by comparing the amount of ganciclovir in plasma to the amount of active ganciclovir that was administered orally. While 1800 mg of valganciclovir were administered orally, this amounts to only 1175 mg of the active moiety, ganciclovir. Therefore, if oral valganciclovir is to be substituted for i.v. ganciclovir in future dosing studies, the concept of therapeutic equivalents (TE) could be employed, where both the ratio of molecular weights ($S = 0.68$) and bioavailability ($F=0.41$) are considered. For example, when calculating a dose of oral valganciclovir that would result in equivalent total body exposure (AUC) to 2.5 mg/kg of i.v. ganciclovir:

$$\text{TE} = \text{Dose}_{\text{i.v. ganciclovir}} \times \frac{1}{F} \times \frac{1}{S}; \quad \text{TE} = \frac{2.5 \text{ mg}}{\text{kg}} \times \frac{1}{0.41} \times \frac{1}{0.68} = 9.0 \text{ mg/kg valganciclovir}$$

Ganciclovir sodium is approved for use in humans and is formulated in 500 mg vials of lyophilized powder for reconstitution with sterile water to a concentration of 50 mg/mL of ganciclovir. For administration to people, injectable ganciclovir is diluted to 10 mg/mL in physiological saline, 5% dextrose, or a Ringer's solution and administered over at least one hour as an infusion.^a The reasoning behind the manufacturer's recommendations for dilution of ganciclovir and the infusion length is unclear and, to the authors' best knowledge, is not available in the refereed literature. However, the manufacturer states that i.v. infusion minimizes peak plasma ganciclovir concentrations and reduces toxicity (CytoveneTM package insert, Laboratories Inc., Nutley, NJ USA). Therefore it is likely that the recommendation for the infusion of diluted ganciclovir solutions in humans is related to safety concerns, such as nephrotoxicity, associated with high peak drug concentrations. Previous studies of i.v. acyclovir use in the horse reported acute adverse effects in 1/6 horses when acyclovir was diluted to 5 mg/mL in normal saline solution and administered as a 15 minute constant rate infusion, resulting in peak plasma acyclovir concentrations of approximately 40 µg/mL (Bentz et al., 2006). In contrast, a subsequent study utilizing a one

^aCytoveneTM package insert; Roche, Laboratories Inc., Nutley, NJ USA

hour infusion duration, with peak plasma acyclovir concentrations of approximately 12 µg/mL, was not associated with toxicity (Maxwell et al., 2008a). Peak concentrations of ganciclovir associated with toxicity have not been well-characterized, but ganciclovir has been administered to rabbits as a single 10 mg/kg i.v. bolus with a resulting peak concentration of approximately 450 µg/mL, without reported side effects (Hedaya & Sawchuk, 1990). The lower potency of acyclovir relative to ganciclovir necessitates the administration of higher acyclovir doses (10 mg/kg) and larger volumes of injectable solution for an equipotent dose, increasing the risk of formulation related adverse effects for acyclovir as compared to ganciclovir. As a result, the intravenous administration of 2.5 mg/kg ganciclovir to horses in the present study resulted in peak plasma ganciclovir concentrations of approximately 15 µg/mL, similar to the peak acyclovir concentrations that are apparently well-tolerated by horses (Wilkins et al., 2005; Bentz et al., 2006; Garre et al., 2007a; Maxwell et al., 2008a). In an effort to increase the practicality of administration to horses, ganciclovir was given as a slow bolus, of approximately 25 mL for a 500 kg horse, over approximately two minutes in the present study. We hypothesized that the lower dose rate of ganciclovir relative to that of acyclovir would permit administration of ganciclovir to horses as a slow bolus, rather than as a prolonged infusion. As an additional consideration, some other drugs administered as an infusion in humans, such as gentamicin, are safely administered as a bolus in horses (Nicolau et al., 1995; Martin-Jimenez et al., 1998). No adverse effects were noted in any horse, suggesting that this protocol was not associated with overt toxicity with single dose ganciclovir administration. It is possible that drug accumulation would occur with multiple doses of ganciclovir in horses, due to the prolonged elimination half-life of ganciclovir in this species. However, such accumulation was predicted to impact the peak concentrations of ganciclovir by a relatively small amount (>10%) due to the pronounced rapid distribution phase of ganciclovir disposition (figure 3). Further testing of the tolerability of multiple doses of ganciclovir in horses are necessary to determine whether side effects or toxicity would occur with the proposed dosing regimen. In all horses in the current study, ganciclovir was administered through an i.v. catheter placed in the jugular vein. Administration of ganciclovir sodium through an i.v. catheter is necessary due to the alkalinity of the formulation (pH = 11) of the drug and the potential caustic effects if extravasated. Adverse effects of ganciclovir administration reported in humans include bone marrow suppression, seizures, nephrotoxicity, and decreased spermatogenesis (Jacobsen & Sifontis, 2010). Therefore, monitoring complete blood counts and chemistry panels in horses receiving ganciclovir therapy is warranted. In human patients with renal compromise, the terminal elimination rate of ganciclovir is prolonged approximately three-fold when compared to patients with normal renal function (Sommadosi et al., 1988). Considering the already prolonged elimination half-life of ganciclovir in the healthy horses utilized in the present study, it is possible that administration of ganciclovir to horses with renal compromise may place them at higher risks for toxicity.

The goals of i.v. ganciclovir therapy included reaching target concentrations of 0.1–0.4 µg/mL within 24 hours, and maintenance at or above those concentrations for greater than 50% of the dosing interval (Tod et al., 2001). Superposition suggested that a loading dose regimen would be necessary to accomplish this goal (Fig. 3). Administration of 2.5 mg/kg

i.v. every 8 hours for the initial 24 hour period followed by 2.5 mg/kg i.v. every 12 hours was predicted to achieve adequate concentrations within 24 hours and maintain ganciclovir plasma levels at or above those levels for the duration of the dosing interval without producing high plasma ganciclovir concentrations that might potentially be associated with toxicity. Mean trough concentrations 24 hours after initiating therapy were predicted to be 0.11 µg/mL and increased steadily on consecutive days, remaining above 0.3 µg/mL by day 2 and above 0.4 µg/mL by the end of day 3. Alternatively, superposition also predicted that an initial dose of 5 mg/kg i.v. would serve as an adequate loading dose and could then be followed by a maintenance regimen of 2.5 mg/kg every 12 hours. However, the safety of this higher initial dose rate was not tested in the current study, and it is unknown whether this larger drug volume and associated higher peak plasma ganciclovir concentrations would be as well-tolerated in horses as was the lower dose rate of 2.5 mg/kg. Therefore, this preliminary study suggests that a dose rate of 2.5 mg/kg of i.v. ganciclovir administered every 8 hours for the first day, followed by 2.5 mg/kg every 12 hours for maintenance would be most suitable for further testing in multiple dose safety and efficacy studies of ganciclovir sodium administration in horses. Recommendations for future testing were based on the principle of superposition, where the drug concentration time course following the administration of multiple doses can be predicted from single dose drug concentration versus time curves (Wang and Ouyang, 1998). However, the use of superposition is based on the key assumption that only linear, or first-order, processes affect metabolism, active renal tubular transport, binding of drug to proteins in plasma and tissues, and other aspects of drug disposition (Thron, 1974). As a consequence, the use of superposition assumes that pharmacokinetic parameters will adhere to linear dispositional processes with multiple doses and the higher drug concentrations that can occur with the multiple dose regimen. Since only one dose of ganciclovir was tested in the present study, dose-proportionality of intravenously administered ganciclovir in horses could not be assessed. Therefore, further testing of the kinetics and safety of multiple dose regimens of intravenously administered ganciclovir should be performed before multiple dose regimens can be recommended for use in equine patients.

Valganciclovir was chosen for oral administration in this study based on human research into the pharmacokinetics of oral ganciclovir and valganciclovir, as well as equine studies examining oral acyclovir and valacyclovir disposition. Human studies have demonstrated the oral bioavailability of ganciclovir to be significantly greater (54–60 %) with administration of valganciclovir as compared with ganciclovir (6–9 %) (Pescovitz, 1999; Acosta et al., 2007). Similarly, equine pharmacokinetic studies have demonstrated improved bioavailability of acyclovir with administration of its prodrug valacyclovir over acyclovir (Maxwell et al., 2008a; Garre et al., 2009b). Therefore, the prodrug valganciclovir was expected to have substantially higher bioavailability in horses as compared to ganciclovir and was selected for testing in the current study. Although the active drug, ganciclovir, is a stable chemical, its prodrug, valganciclovir, is easily hydrolyzed at its ester bond to form valine and ganciclovir. Valganciclovir has been shown to be stable for up to 35 days in solutions with a pH of 3.6–3.8, but unstable at a neutral to basic pH (Henkin et al., 2003; Stefanidis & Brandl, 2005). In the current study valganciclovir was administered as a paste consisting of lemon juice, syrup, and flour and was administered immediately after

preparation. As the formulation pH and the drug stability of this mixture were not determined in this study, stability studies may be indicated if drug is to be prepared in an aqueous solution and stored, rather than immediately administered, in any further studies of valganciclovir in horse.

Although the bioavailability of valganciclovir was moderate (41 %) and similar to the bioavailability of valacyclovir in horses, variability among horses was substantial, with a coefficient of variation of 50%. Superposition of ganciclovir data from the administration of 1800 mg valganciclovir orally administered every 12 hours predicted that trough concentrations would fail to reach target levels and remain at or above those levels for greater than 50 % of the dosing interval. Superposition predictions based on a dose of 3600 mg every 12 hours were able to satisfy the above criteria. The mean trough concentration predicted at 24 hours with this regimen was 0.25 µg/mL. By day 4, trough concentrations were predicted to remain above 0.3 µg/mL (Fig. 4). However, this prediction assumes linear pharmacokinetics of valganciclovir which has not been demonstrated in horses and is not the case in humans (Wiltshire et al., 2005; Acosta et al., 2007). In addition, drug costs associated with administering 3600 mg of valganciclovir twice a day for one week approach \$5000 and therefore may be cost prohibitive. Additional research is needed before oral therapy with valganciclovir can be recommended in horses, due to the variability of pharmacokinetic parameters, the moderate bioavailability, and its current high cost.

Based on the results of this study, antiviral therapy with ganciclovir is recommended with a loading regimen of 2.5 mg/kg every 8 hours for 24 hours, followed by maintenance therapy at a dose rate 2.5 mg/kg every 12 hours. Current drug costs for seven days of i.v. ganciclovir sodium therapy are approximately \$2200 (\$150/dose). However, the cost of ganciclovir may change in the future as the drug is not under patent protection. Further research utilizing experimental models or clinical trials is warranted to determine the efficacy of ganciclovir in horses infected with EHV-1

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References

- Acosta EP, Brundage RC, King JR, Sanchez PJ, Sood S, Agrawal V, Homans J, Jacobs RF, Lang D, Romero JR, Griffin J, Cloud G, Whitley R, Kimberlin DW. Ganciclovir population pharmacokinetics in neonates following intravenous administration of ganciclovir and oral administration of a liquid valganciclovir formulation. *Clinical Pharmacology and Therapeutics*. 2007; 81:867–872.
- Bentz BG, Maxwell LK, Erkert RS, Royer CM, Davis MS, MacAllister CG, Clarke CR. Pharmacokinetics of acyclovir after single intravenous and oral administration to adult horses. *Journal of Veterinary Internal Medicine*. 2006; 20:589–594. [PubMed: 16734094]
- Chu F, Kiang CH, Sung ML, Huang B, Reeve RL, Tarnowski T. A rapid, sensitive HPLC method for the determination of ganciclovir in human plasma and serum. *Journal of Pharmaceutical and Biomedical Analysis*. 1999; 21:657–667. [PubMed: 10701434]
- Edington N, Bridges CG, Patel JR. Endothelial cell infection and thrombosis in paralysis caused by equid herpesvirus-1: equine stroke. *Archives of Virology*. 1986; 90:111–124. [PubMed: 3015074]

- Fletcher C, Sawchuk R, Chinnock B, de Miranda P, Balfour HH Jr. Human pharmacokinetics of the antiviral drug DHPG. *Clinical Pharmacology and Therapeutics*. 1986; 40:281–286. [PubMed: 3017630]
- Garré B, Baert K, Nauwynck H, Deprez P, De Backer P, Croubels S. Multiple oral dosing of valacyclovir in horses and ponies. *Journal of Veterinary Pharmacology and Therapeutics*. 2009a; 32:207–212. [PubMed: 19646083]
- Garré B, Gryspeerdt A, Croubels S, De Backer P, Nauwynck H. Evaluation of orally administered valacyclovir in experimentally EHV1-infected ponies. *Veterinary Microbiology*. 2009b; 135:214–221. [PubMed: 18986780]
- Garré B, Shebany K, Gryspeerdt A, Baert K, van der Meulen K, Nauwynck H, Deprez P, De Backer P, Croubels S. Pharmacokinetics of acyclovir after intravenous infusion of acyclovir and after oral administration of acyclovir and its prodrug valacyclovir in healthy adult horses. *Antimicrobial Agents and Chemotherapy*. 2007a; 51:4308–4314. [PubMed: 17846132]
- Garré B, van der Meulen K, Nugent J, Neyts J, Croubels S, De Backer P, Nauwynck H. In vitro susceptibility of six isolates of equine herpesvirus 1 to acyclovir, ganciclovir, cidofovir, adefovir, PMEDAP and foscarnet. *Veterinary Microbiology*. 2007b; 122:43–51. [PubMed: 17276631]
- Goodman LB, Loregian A, Perkins GA, Nugent J, Buckles EL, Mercorelli B, Kydd JH, Palu G, Smith KC, Osterrieder N, Davis-Poynter N. A point mutation in a herpesvirus polymerase determines neuropathogenicity. *PLoS Pathog*. 2007; 3:e160. [PubMed: 17997600]
- Hedaya MA, Sawchuk RJ. A sensitive and specific liquid-chromatographic assay for determination of ganciclovir in plasma and urine and its application to pharmacokinetic studies in the rabbit. *Pharmaceutical Research*. 1990; 7:1113–1118. [PubMed: 2293208]
- Henkin CC, Griener JC, Ten Eick AP. Stability of valganciclovir in extemporaneously compounded liquid formulations. *American Journal of Health-System Pharmacy*. 2003; 60:687–690. [PubMed: 12701551]
- Jacobsen T, Sifontis N. Drug interactions and toxicities associated with the antiviral management of cytomegalovirus infection. *American Journal of Health-System Pharmacy*. 2010; 67:1417–1425. [PubMed: 20720240]
- Jacobson MA, de Miranda P, Cederberg DM, Burnette T, Cobb E, Brodie HR, Mills J. Human pharmacokinetics and tolerance of oral ganciclovir. *Antimicrobial Agents and Chemotherapy*. 1987; 31:1251–1254. [PubMed: 2820301]
- Krasny HC, de Miranda P, Blum MR, Elion GB. Pharmacokinetics and bioavailability of acyclovir in the dog. *Journal of Pharmacology and Experimental Therapeutics*. 1981; 216:281–288. [PubMed: 7463350]
- Kydd JH, Townsend HG, Hannant D. The equine immune response to equine herpesvirus-1: the virus and its vaccines. *Veterinary Immunology and Immunopathology*. 2006; 111:15–30. [PubMed: 16476492]
- Lunn DP, Davis-Poynter N, Flaminio MJ, Horohov DW, Osterrieder K, Pusterla N, Townsend HG. Equine herpesvirus-1 consensus statement. *Journal of Veterinary Internal Medicine*. 2009; 23:450–461. [PubMed: 19645832]
- Martin-Jimenez T, Papich MG, Riviere JE. Population pharmacokinetics of gentamicin in horses. *American Journal of Veterinary Research*. 1998; 59:1589–1598. [PubMed: 9858412]
- Maxwell LK, Bentz BG, Bourne DW, Erkert RS. Pharmacokinetics of valacyclovir in the adult horse. *Journal of Veterinary Pharmacology and Therapeutics*. 2008a; 31:312–320. [PubMed: 18638291]
- Maxwell, LK.; Bentz, BG.; Gilliam, LL.; Ritchey, JW.; Eberle, RW.; Holbrook, TC.; McFarlane, D.; Rezabek, GB.; MacAllister, CG.; Goad, CL.; Allen, GP. In: Werner, HW., editor. Efficacy of valacyclovir against clinical disease after EHV-1 challenge in aged mares; Proceedings of the 54th Annual Convention of the American Association of Equine Practitioners; San Diego, California, USA. 6–10 December 2008; Lexington: American Association of Equine Practitioners (AAEP); 2008b. p. 198-199.
- Nicolau DP, Freeman CD, Belliveau PP, Nightingale CH, Ross JW, Quintiliani R. Experience with a once-daily aminoglycoside program administered to 2,184 adult patients. *Antimicrobial Agents and Chemotherapy*. 1995; 39:650–655. [PubMed: 7793867]

- Nugent J, Birch-Machin I, Smith KC, Mumford JA, Swann Z, Newton JR, Bowden RJ, Allen GP, Davis-Poynter N. Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. *Journal of Virology*. 2006; 80:4047–4060. [PubMed: 16571821]
- Perkins GA, Goodman LB, Tsujimura K, Van de Walle GR, Kim SG, Dubovi EJ, Osterrieder N. Investigation of the prevalence of neurologic equine herpes virus type 1 (EHV-1) in a 23-year retrospective analysis (1984–2007). *Veterinary Microbiology*. 2009; 139:375–378. [PubMed: 19615831]
- Pescovitz MD. Oral ganciclovir and pharmacokinetics of valganciclovir in liver transplant recipients. *Transplant Infectious Disease*. 1999; 1(Suppl 1):31–34. [PubMed: 11565585]
- Pollina GF, Zagotto G, Maritan P, Iacopetti I, Busetto R. Pharmacokinetics of gallium nitrate after oral administration in adult horses - pilot study. *Journal of Veterinary Pharmacology and Therapeutics*. 2011
- Saxegaard F. Isolation and identification of equine rhinopneumonitis virus (equine abortion virus) from cases of abortion and paralysis. *Nordisk Veterinaermedicin*. 1966; 18:504–512.
- Serabe BM, Murry DJ, Dauser R, Nuchtern J, Durfee J, McGuffey L, Berg S, Blaney SM. Plasma and CSF pharmacokinetics of ganciclovir in nonhuman primates. *Cancer Chemotherapy and Pharmacology*. 1999; 43:415–418. [PubMed: 10100598]
- Sommadossi JP, Bevan R, Ling T, Lee F, Mastre B, Chaplin MD, Nerenberg C, Koretz S, Buhles WC Jr. Clinical pharmacokinetics of ganciclovir in patients with normal and impaired renal function. *Reviews of Infectious Diseases*. 1988; 10(Suppl 3):S507–514. [PubMed: 2847287]
- Stefanidis D, Brandl M. Reactivity of valganciclovir in aqueous solution. *Drug Development and Industrial Pharmacy*. 2005; 31:879–884. [PubMed: 16305999]
- Thron CD. Linearity and superposition in pharmacokinetics. *Pharmacological Reviews*. 1974; 26:3–31. [PubMed: 4133328]
- Tod M, Lokiec F, Bidault R, De Bony F, Petitjean O, Aujard Y. Pharmacokinetics of oral acyclovir in neonates and in infants: a population analysis. *Antimicrobial Agents and Chemotherapy*. 2001; 45:150–157. [PubMed: 11120958]
- Tsujimura K, Yamada M, Nagata S, Yamanaka T, Nemoto M, Kondo T, Kurosawa M, Matsumura T. Pharmacokinetics of penciclovir after oral administration of its prodrug famciclovir to horses. *Journal of Veterinary Medical Science*. 2010; 72:357–361. [PubMed: 19959884]
- Wang W, Ouyang SP. The formulation of the principle of superposition in the presence of non-compliance and its applications in multiple dose pharmacokinetics. *Journal of Pharmacokinetics and Biopharmaceutics*. 1998; 26:457–469. [PubMed: 10214562]
- Wilkins, PA.; Henninger, R.; Reed, SM.; Piero, Fd; del Piero, F. Acyclovir as treatment for EHV-1 myeloencephalopathy. *Proceedings of the 49th Annual Convention of the American Association of Equine Practitioners*; New Orleans, Louisiana, USA. 21–25 November 2003; Lexington: American Association of Equine Practitioners (AAEP); 2003. p. 394-396.
- Wilkins PA, Papich M, Sweeney RW. Pharmacokinetics of acyclovir in adult horses. *Journal of Veterinary Emergency and Critical Care*. 2005; 15:174–178.
- Wilson KE, Wilcke JR, Crisman MV, Ward DL, McKenzie HC, Scarratt WK. Comparison of serum iothexol clearance and plasma creatinine clearance in clinically normal horses. *American Journal of Veterinary Research*. 2009; 70:1545–1550. [PubMed: 19951127]
- Wiltshire H, Hirankarn S, Farrell C, Paya C, Pescovitz MD, Humar A, Dominguez E, Washburn K, Blumberg E, Alexander B, Freeman R, Heaton N. Pharmacokinetic profile of ganciclovir after its oral administration and from its prodrug, valganciclovir, in solid organ transplant recipients. *Clinical Pharmacokinetics*. 2005; 44:495–507. [PubMed: 15871635]
- Winston DJ, Baden LR, Gabriel DA, Emmanouilides C, Shaw LM, Lange WR, Ratanatharathorn V. Pharmacokinetics of ganciclovir after oral valganciclovir versus intravenous ganciclovir in allogeneic stem cell transplant patients with graft-versus-host disease of the gastrointestinal tract. *Biology of Blood and Marrow Transplantation*. 2006; 12:635–640. [PubMed: 16737936]
- Zhao W, Boudouin V, Zhang D, Deschenes G, Le Guellec C, Jacqz-Aigrain E. Population pharmacokinetics of ganciclovir following administration of valganciclovir in paediatric renal transplant patients. *Clinical Pharmacokinetics*. 2009; 48:321–328. [PubMed: 19566115]

Fig 1. Observed (mean $\pm$ s.d.) and predicted (solid line) plasma ganciclovir concentrations versus time following a single 2.5 mg/kg dose of ganciclovir sodium administered as a slow intravenous bolus to six horses.

Fig 2. Observed (mean $\pm$ s.d.) and predicted (solid line) plasma ganciclovir concentrations versus time following a single 1800 mg dose of valganciclovir administered orally to six horses.

Fig 3.

Mean (solid line) and 95% confidence interval (dashed lines) for plasma ganciclovir concentrations predicted to result from the intravenous administration of ganciclovir sodium at a loading dose of 2.5 mg/kg every 8 hr for 1 day, followed by a maintenance dose of 2.5 mg/kg every 12 hr. Dotted lines represent the targeted trough concentrations.

Fig 4.

Mean (solid line) and 95% confidence interval (dashed lines) for plasma ganciclovir concentrations predicted to result from the oral administration of valganciclovir at a loading dose of 3600 mg every 8 hr for 1 day, followed by a maintenance dose of 3600 mg every 12 hr. Dotted lines represent the targeted trough concentrations.

Table 1

Pharmacokinetic parameters determined following a single 2.5mg/kg dose of ganciclovir administered as a slow intravenous bolus to six horses.

Pharmacokinetic parameters	
C_0 (µg/ml)	15 ± 4
A (µg/ml)	5.9 ± 1.5
B (µg/ml)	0.31 ± 0.10
C (µg/ml)	0.031 ± 0.009
$t_{1/2(\lambda_1)}$ (hr)	$0.093 \pm 0.024^*$
$t_{1/2(\lambda_2)}$ (hr)	$1.5 \pm 0.5^*$
$t_{1/2(\lambda_3)}$ (hr)	$72 \pm 9^*$
k_{10} (hr ⁻¹)	1.3 ± 0.2
k_{12} (hr ⁻¹)	3.0 ± 0.5
k_{13} (hr ⁻¹)	2.7 ± 1.4
k_{31} (hr ⁻¹)	0.031 ± 0.012
V_c (l/kg)	0.17 ± 0.04
$V_{d(ss)}$ (l/kg)	15 ± 2.0
Cl (ml/min·kg)	3.6 ± 0.7
AUC _{total} (µg·hr/ml)	11.9 ± 2.3
MRT (hr)	71 ± 12
% contribution A_1/λ_1	17 ± 3
% contribution A_2/λ_2	16 ± 6
% contribution A_3/λ_3	67 ± 8

Values are expressed as the mean or *harmonic mean $\pm$ s.d. C_0 = serum drug concentration at time 0; A = coefficient of rapid distribution phase; B = coefficient of slow distribution phase; C = coefficient of elimination phase; $t_{1/2(\lambda_1)}$ = rapid distributional half-life; $t_{1/2(\lambda_2)}$ = slow distributional half-life; $t_{1/2(\lambda_3)}$ = terminal elimination phase half-life; k_{10} = first-order rate constant for ganciclovir elimination from the central compartment; other intercompartmental rate constants follow similar nomenclature; V_c = apparent volume of the central compartment; $V_{d(ss)}$ = apparent volume of distribution at steady state; Cl = total body clearance; AUC_{total} = Area under the plasma ganciclovir concentration versus time curve, extrapolated to infinity; MRT = mean residence time, % contribution $A_1/\lambda_1 = (A_1/\lambda_1 / AUC_{total}) \times 100$.

Table 2

Ganciclovir pharmacokinetic parameters resulting from the oral administration of 1800 mg of valganciclovir to six horses.

Pharmacokinetic parameters	
$C_{\max}$ (µg/ml)	0.58 ± 0.37
T_{lag} (hr)	0.17 ± 0.06
$T_{\max}$ (hr)	1.3 ± 0.3
$V_{d(\text{area})}/F$ (L/kg)	49 ± 20
$t_{1/2 \text{ abs}}$ (hr)	$0.66 \pm 0.26^*$
$t_{1/2(\alpha)}$ (hr)	$0.69 \pm 0.30^*$
$t_{1/2(\beta)}$ (hr)	$43 \pm 20^*$
AUC_{total} (µg·hr/ml)	4.4 ± 2.7
MRT (hr)	83 ± 69
F (%)	41 ± 20

Values are expressed as the mean or *harmonic mean $\pm$ s.d. $C_{\max}$ = maximum observed plasma drug concentration; T_{lag} = lag time; $T_{\max}$ = time at which $C_{\max}$ was observed; MRT = mean residence time; $V_{d(\text{area})}/F$ = apparent volume of distribution during the terminal phase/bioavailability; $t_{1/2(\text{abs})}$ = absorption half-life; $t_{1/2(\alpha)}$ = rapid distributional phase half-life; $t_{1/2(\beta)}$ = elimination phase half-life; AUC_{total} = area under the plasma concentration-time curve extrapolated to infinity, F = Bioavailability.