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Efficacy of the early administration of valacyclovir hydrochloride for the treatment of neuropathogenic equine herpesvirus type-1 infection in horses

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Abstract

OBJECTIVE—To determine whether prophylactic administration of valacyclovir hydrochloride versus initiation of treatment at the onset of fever would differentially protect horses from viral replication and clinical disease attributable to equine herpesvirus type-1 (EHV-1) infection.

ANIMALS—18 aged mares.

PROCEDURES—Horses were randomly assigned to receive an oral placebo (control), treatment at detection of fever, or prophylactic treatment (initiated 1 day prior to viral challenge) and then inoculated intranasally with a neuropathogenic strain of EHV-1. Placebo or valacyclovir was administered orally for 7 or 14 days after EHV-1 inoculation or detection of fever (3 horses/group). Effects of treatment on viral replication and clinical disease were evaluated. Plasma acyclovir concentrations and viremia were assessed to determine inhibitory concentrations of valacyclovir.

RESULTS—Valacyclovir administration decreased shedding of virus and viremia, compared with findings for control horses. Rectal temperatures and clinical disease scores in horses that received valacyclovir prophylactically for 2 weeks were lower than those in control horses. The severity of but not the risk for ataxia was decreased by valacyclovir administration. Viremia was decreased

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when steady-state trough plasma acyclovir concentrations were > 0.8 μg/mL, supporting the time-dependent activity of acyclovir.

CONCLUSIONS AND CLINICAL RELEVANCE—Valacyclovir treatment significantly decreased viral replication and signs of disease in EHV-1–infected horses; effects were greatest when treatment was initiated before viral inoculation, but treatment was also effective when initiated as late as 2 days after inoculation. During an outbreak of equine herpesvirus myeloencephalopathy, antiviral treatment may be initiated in horses at various stages of infection, including horses that have not yet developed signs of viral disease.

Infection with EHV-1 is associated with different signs of disease in different classes of horses; young horses are likely to develop upper respiratory tract signs, pregnant mares may abort their fetuses, and mature horses are most susceptible to neurologic disease. Neurologic signs of disease, termed EHM, are more likely to develop in aged (> 20 years old) horses than in younger (< 15 years old) horses and are rare in immature horses.[1] High-profile outbreaks of EHV-1 infection with resulting EHM have stimulated interest in the safe and effective use of antiherpetic drugs in horses.[2] Because viral infection does not contemporaneously affect all horses in a herd, it is expected that horses in a barn during the early stages of an outbreak will be at different stages of EHV-1 infection. Some horses will have already been infected with EHV-1 at the time that an outbreak is recognized, whereas others may have not yet been exposed. Because vaccination does not uniformly protect horses from EHV-1-induced neurologic disease, antiherpetic drug treatment could protect susceptible horses from severe disease during an outbreak.[2,3] However, for other herpesviruses, the timing of administration of antiherpetic treatment affects outcomes, with early treatment associated with better outcomes.[4]

Although several antiviral drugs have activity against EHV-1 in vitro and have been used in viral outbreaks, evidence supporting the efficacy of these drugs in vivo has been lacking.[2,5,a] Without scientific evidence of the efficacy of antiherpetic drugs against EHV-1 infection in live animals, clinicians cannot confidently recommend the use of these drugs in horses.

Several groups have recently demonstrated that orally administered valacyclovir is absorbed well in adult horses.[6,7] Valacyclovir is a prodrug for the antiherpetic drug acyclovir, which has efficacy against several strains of EHV-1 in vitro. As a consequence of its favorable pharmacokinetic profile and in vitro efficacy,[8–10,a,b] we hypothesized that oral treatment with valacyclovir could protect horses from development of clinical signs associated with EHV-1 infection. However, the timing of treatment was expected to be important, given that successful antiherpetic treatment in other species appears to be dependent on administration of these drugs early after viral inoculation.[4] The objective of the study reported here was to determine whether prophylactic administration of valacyclovir versus initiation of treatment

[a]Wilkins P. Acyclovir in the treatment of EHV-1 myeloencephalopathy (abstr), in *Proceedings.* 22nd Annu Meet Am Coll Vet Intern Med 2004;170-172.

[b]Henninger R. Epidemic neurologic disease due to equine herpesvirus 1: The University of Findlay English Equestrian Center (abstr), in *Proceedings*. Int Vet Emerg Crit Care Symp 9th Annu Meet 2003;621-624.

at the onset of fever would differentially protect horses from viral replication and clinical disease attributable to EHV-1 infection.

Materials and Methods

Animals

Eighteen mares that appeared to be light horse breeds[11] were included in the study. This sample size was selected to detect a 90% reduction (compared with control findings) in maximum viremia for 2 drug treatment groups with a power of 0.90 ($\alpha = 0.05$) on the basis of results of a previous study[12] involving challenge with the same strain of EHV-1 used in the present study. The horses were university owned and selected on the basis of registration papers or dental examination consistent with an age of > 20 years, ataxia scores of grade 1 or 0 (on a modified scale of 0 to 5 [Appendix 1][13,14]), and seronegativity for anti-EHV-1 antibodies in serum as determined by an EHV-1 type-specific ELISA.15,[c] Serum anti-EHV-4 antibody titers were not assessed. Mares were not pregnant and were each deemed healthy on the basis of results of a physical examination, CBC, and serum biochemical analysis. The study was approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Experimental design

Horses were randomly allocated by draw from a hat into 1 of 3 groups: no drug treatment (control group [n = 6]), prophylactic valacyclovir hydrochloride administration (prophylactic treatment group [6]), and valacyclovir hydrochloride administration after detection of fever (febrile treatment group [6]). The study was performed during 2 separate experimental periods with 9 horses per period. In the first period, valacyclovir was administered to horses either prophylactically (ie, treatment initiated 1 day prior to EHV-1 challenge and continued for 1 week after viral inoculation; n = 3) or after detection of fever (treatment continued for 1 week total for each horse; 3). In the second period, valacyclovir was administered to horses either prophylactically (treatment continued for 2 weeks after viral inoculation; n = 3) or after detection of fever (treatment continued for 2 weeks for each horse; 3). Three control horses were also included in each period. Both periods were conducted in a single winter season, with a 6-week interval between inoculation of horses in the first and second periods.

On day 0 in either period, horses in the control and 2 treatment groups were inoculated intranasally via a fenestrated 30.5-cm-long rubber catheter with 10^7 PFU of EHV-1 (strain Findlay OH 2003 [T953]). This is a neuropathogenic strain of EHV-1[d] that was recovered from horses during an outbreak of EHM.[b]

Experimental treatments

In the first and second experimental periods, horses assigned to the control group received a daily oral placebo treatment (25 mL of flour mixed with 25 mL of corn syrup) for 1 and 2 weeks, respectively. In either experimental period, administration of valacyclovir

[c]Boehringer Ingelheim Svanova, Uppsala, Sweden
[d]Provided by one of the investigators (GPA).

hydrochloride[e] to the horses assigned to the prophylactic treatment group began 1 day before challenge with EHV-1; the viral inoculation was performed on the second day of the loading dose regimen (day 0). In either experimental period, rectal temperature of each horse assigned to the febrile treatment group was monitored twice daily from day -2, and administration of valacyclovir began when the horse's temperature was ≥ 38.5°C. In the valacyclovir treatment groups, the drug was administered at a loading dose rate of 27 mg/kg, PO, every 8 hours for 2 days, followed by a maintenance dose of 18 mg/kg, PO, every 12 hours for the duration of the assigned treatment period.[7] Valacyclovir tablets were crushed with a coffee grinder once daily, with each resulting dose stored in individual, labeled, air-tight plastic containers until administration. Valacyclovir powder was not mixed with corn syrup until immediately prior to oral administration by syringe. Because doses were individualized to the body weight of each horse, the amount of corn syrup added varied, with 1 part valacyclovir powder mixed with 1 part corn syrup to form a thin paste.

Data collection

For all horses during both experimental periods, rectal temperature was determined twice daily from -2 through 14 days after EHV-1 inoculation, and each measurement was used as a separate data point. From days 2 through 14 after EHV-1 inoculation, a daily clinical score for each horse was generated by use of an adaptation of a previously reported method[16] (Appendix 2). Subjective clinical data were collected daily by an investigator (LLG) who was unaware of the horse's group assignment. The daily clinical score was the sum of the scores for the data that were collected daily (attitude, lymph node size, and nasal and ocular discharge) and the highest of the 2 scores that each horse received for the data that were collected twice daily (appetite, respiratory rate, and heart rate). The mean daily clinical score for each horse was the sum of its daily clinical scores from day 2 through day 14 divided by the number of days that the horse remained in the study during that time period. Neurologic examinations were performed daily by a board-certified equine internist (LLG) at -2 days and from 2 through 14 days after EHV-1 inoculation. Neurologic examinations were also recorded on videotape, digitized, randomized with respect to subject and time, and then evaluated by a second board-certified equine internist (TCH) who was also unaware of the horse's group assignment. Ataxia was graded on a modified 6-point ataxia scale (Appendix 1).[13,14] Only days in which both independent evaluators agreed on the ataxia score were retained for further analysis. Change in ataxia was determined as the difference between the consensus ataxia score and the baseline ataxia score.

From each horse, a blood sample (10 mL each) was collected into vacuum tubes containing heparin[f] before inoculation with EHV-1 on day 0 and every day through 14 days after inoculation. Peripheral blood mononuclear cells were isolated from the blood samples by differential centrifugation with a density gradient medium12,[g] and stored at −70°C until analysis. Viremia was quantified by a qPCR assay with a previously described method.[17] Nasopharyngeal swab samples were collected on the day before EHV-1 inoculation (day -1), and once daily from day 1 until 14 days after inoculation. Each swab was placed in 3.5 mL

[e]Valtrex, GlaxoSmithKline, Mississauga, ON, Canada.
[f]Vacutainer, BD, Franklin Lakes, NJ.
[g]Ficoll-hypaque, Amersham Biosciences, Piscataway, NJ.

of Dulbecco modified Eagle minimal essential medium, supplemented with penicillin, streptomycin, amphotericin B,[h] and L-glutamine[i] and containing 2% heat inactivated and irradiated fetal bovine serum,[j] (2% supplemented Dulbecco modified Eagle minimal essential medium), and stored at −80°C. For the virus assay, each sample was clarified by centrifugation and filtered; 100 μL of the sample was then added to confluent Madin-Darby bovine kidney cells in 6-well plates for 1 hour at 37°C. The supernatant was then aspirated and replaced with 500 μL of 2% supplemented Dulbecco modified Eagle minimal essential medium and maintained at 37°C. Cells with signs of cytopathogenic effects were scraped from wells into the medium and frozen at −80°C for viral quantitation. Virus was released from the cells by 3 freeze-thaw cycles; the samples were clarified by centrifugation, and 10-fold dilutions were prepared. Diluted samples (100 μL) were inoculated in duplicate onto confluent Madin-Darby bovine kidney cell monolayers in 6-well plates and incubated at 37°C for 72 hours; plaques were then counted to quantitate PFU. Viral DNA in nasal swabs was also determined by qPCR assay.[18] Additional heparinized and EDTA[f] anticoagulated blood samples (10 mL each) from all treated and control horses were submitted for CBC and plasma biochemical analysis at weekly intervals (days 7 and 14).

Horses from all treatment groups and periods were euthanized by means of an IV overdose injection of pentobarbital and necropsied on day 15 after inoculation, except for 2 horses that were euthanized and necropsied on days 11 and 14 because of progression of neurologic disease. A complete gross necropsy examination was performed on each horse during which fresh tissue samples were collected for viral load determinations by qPCR assay and for formalin fixation and subsequent histologic examination and immunohistochemical analysis for viral antigen. A CSF sample was also collected from the atlanto-occipital space of each treated and control horse. Fresh tissue samples were collected, including lymph nodes (mandibular or retropharyngeal lymph node, with the largest lymph node collected), brain (cerebral cortex and cerebellum), spinal cord (C5, T6, and L1 segments), auditory tube diverticula (guttural pouches), nasal turbinates, pharynx, liver, lungs, and kidneys. Tissue samples were kept frozen and maintained at −80°C during shipment and prior to analysis. Viral load in samples of each tissue was quantified by qPCR assay.[17] Viral loads were determined by use of plasmids to generate slope and y-intercept values. Quantification cycle values were included in a formula to calculate the number of copies of each gene. A specimen (0.1 g [approx the size of a grain of rice]) of each type of tissue was ground in lysis buffer and extracted on a semiautomated system. This sampling technique has been previously found to yield similar viral loads from multiple sections of the same organ (trigeminal ganglion and mandibular lymph node) in horses infected with EHV-1.[19] Tissue was standardized to the number of sampled cells with a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase, and reported as the number of target genes per million eukaryotic cells. Viral load in CSF samples was also quantified by qPCR assay. For immunohistochemical analysis, the primary antibody was an anti-herpesvirus antibody raised in goats,[k] and the secondary antibody was a rabbit anti-goat antibody.[l]

[h]·Fungizone, Bristol-Myers Squibb Co, New York, NY.
[i]·Sigma-Aldrich, St Louis, Mo.
[j]·Altas Biologicals, Fort Collins, Colo.
[k]·VMRD Inc, Pullman, Wash.
[l]·Zymed Laboratories, South San Francisco, Calif.

The remainder of the brain and spinal cord (in its entirety) along with the other tissues was fixed in formalin. After formalin fixation, the brain was sectioned into 0.5-cm-thick layers from the rostral to the caudal portion of the brainstem. Representative sections of the brain were collected from the cerebrum rostral to the cruciate sulcus, frontal portion of the cerebrum at the level of the corpus callosum and internal capsule, cerebrum at the level of the midbrain, hippocampus, and cerebellum and pons. Similarly, the spinal cord was transected at every segment just caudal to the exit of the ventral spinal roots. The CNS tissues were evaluated and scored independently by 2 pathologists (JWR and GBR). This histologic lesion score was based on the level of inflammation within the meninges, perivascular cuffing or vascular lesions, and spongiosis and was derived by use of a histologic scoring system adapted from that previously used in mice with Theiler encephalomyelitis.[20] Any sections found to be abnormal were processed for immunohistochemical analysis to detect herpesvirus antigen.

Safety and pharmacokinetic and pharmacodynamic parameters after valacyclovir administration

A sample of blood (10 mL) was collected from each horse at predetermined time points (0 [immediately before drug administration], 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 8, and 12 hours) after the administration of valacyclovir on the first and seventh days after EHV-1 inoculation in all valacyclovir-treated horses, and additionally at those time points 14 days after inoculation in the horses that received 2 weeks of treatment. To determine trough plasma valacyclovir concentrations, blood samples (10 mL each) were collected in the morning, just before the next dose of valacyclovir, on days 2 through 6 from all valacyclovir-treated horses, and additionally on days 8, 10, 12, 13, 14, and 15. On day 15 after EHV-1 inoculation, the trough blood sample was obtained immediately before euthanasia by IV overdose injection of pentobarbital.

Cerebrospinal fluid samples from the atlanto-occipital space and bone marrow aspirates from the sternum were obtained immediately after euthanasia from the 6 horses that received valacyclovir treatment for 2 weeks. The CSF and bone marrow were immediately examined microscopically for abnormalities by a board-certified clinical pathologist (JM). Plasma and CSF concentrations of acyclovir were determined by high-performance liquid chromatography, as previously described.[7] The accuracy of the plasma quantitative assay for acyclovir concentration (at 0.05, 0.5, and 5 μg/mL) was 94%, 94%, and 96%, respectively, whereas the coefficient of variation was 13%, 6%, and 4%, respectively. The CSF samples were assayed for acyclovir concentration with the same protein precipitation method and internal standard as those used for the plasma samples, and the assay performance was similar. The calibration curve was prepared at acyclovir concentrations from 0.04 to 2 μg/mL in CSF that was obtained at necropsy from the unmedicated control horses. The accuracy of the assay for acyclovir concentration (0.05 and 0.5 μg/mL) in CSF was 96% and 102%, respectively, whereas the coefficient of variation was 4% and 3%, respectively.

Compartmental analysis was performed with commercial software[e] to determine pharmacokinetic parameters for acyclovir in plasma over the duration of valacyclovir administration. The values of $C_{min(ss)}$, $C_{max(ss)}$, area under the plasma drug concentration-

time curve from 0 to 8 hours after the first dose, and $AUC_{\tau(ss)}$ were determined. The concentration of acyclovir, penciclovir, and ganciclovir required to inhibit EHV-1 strain T953 plaque formation by 50% (IC_{50}) was determined in this study in vitro (in triplicate) in equine fetal lung cells by a sigmoidal inhibitory maximum effect (E_{max}) model with commercial software, as previously described.21,[m] The in vivo IC_{50} was also assessed by calculation of the area under the curve of viremia from the qPCR results for EHV-1 DNA in PBMCs over the 14-day study period.[12] Stepwise multiple linear regression analysis[n] was used to determine whether $C_{min(ss)}$, $C_{max(ss)}$, $AUC_{\tau(ss)}$, or the percentage of the dosing interval > IC_{50} affected viremia (expressed as $\log_{10}$ AUC of EHV-1 copies•d/10^6 cells). The pharmacokinetic parameter that best predicted the reduction in viremia was used to determine the in vivo IC_{50} from the sigmoidal inhibitory E_{max} model.[m]

Statistical analysis

The data were analyzed as a 2 × 2 factorial (2 drug treatment groups × 2 dosing periods) plus a control group. For the responses measured over time, a repeated-measures analysis was performed with linear mixed-models methods. When a significant effect was detected, post hoc comparisons of the data for the active treatment groups versus data for the control group were examined. All tests of significance were conducted at the nominal level of α = 0.05. Data were analyzed with softwares.[o] There was no significant difference in ataxia score between the proportion of horses treated with valacyclovir prophylactically or at the onset of fever, or between experimental periods. Thus, horses for which treatment with valacyclovir was initiated at -1 to 2 days after EHV-1 inoculation and for which treatment was administered for 1 or 2 weeks were combined into a single drug treatment group for comparison with the proportion of control horses that developed variable severities of ataxia by a Fisher exact test. However, to allow the visualization of data for each group, a graph of ataxia over time was composed with separation of treatment groups. Other graphs and tables were composed with the groups separated as needed to demonstrate significant differences.

Results

One horse in the febrile treatment groups had intermittent mucopurulent nasal discharge during the study and evidence of chronic bronchopneumonia at necropsy, with the onset of lower respiratory tract disease estimated to have substantially preceded inoculation with EHV-1. Although this horse was allocated to the febrile treatment group and received valacyclovir for 2 weeks, only the drug safety data were analyzed because of the preexisting bronchopneumonia. This horse had few clinical signs specific to EHV-1 infection, although intermittent fever and mucopurulent nasal discharge were noted.

Pathological findings

Histologic lesions were not present in the examined brain sections from any of the control or treated horses. Histologic lesions in the spinal cord were not consistently related to herpesvirus infection, but rather were more commonly lesions related to age (eg, dural

[m]WinNonlin Professional, Pharsight Corp, Mountain View, Calif.
[n]SigmaStat, version 3.0, SPSS, Chicago, Ill.
[o]SAS, version 9.4, SAS Institute Inc, Cary, NC.

mineralization, dural ossification, and rare axonal loss). There were 2 single foci of vasculitis consistent with herpesvirus infection located in the C2-C3 segment of one horse and in the cervical intumescence of another horse, with one of these horses being a control horse and the other treated prophylactically with valacyclovir for 1 week. These sections, along with representative sections of nonlesional spinal cord, underwent immunohistochemical analysis to detect herpesvirus antigen; no staining was evident in any of the sections. Clinically important lesions were not seen in any visceral tissues examined.

Viral loads

The highest concentration of infectious virus was recovered from nasal swab samples at day 1 after EHV-1 inoculation in all horses (Figure 1). Virus was isolated as late as 9 days after EHV-1 inoculation, but because viral isolation was achieved in only 2 horses on day 5 and in 1 horse on days 6 to 9 after EHV-1 inoculation, statistical comparisons were restricted to data obtained on the first 4 days after viral challenge. By means of qPCR assay, EHV-1 was detected through 14 days after EHV-1 inoculation (Figure 2). Although the mean nasal EHV-1 load as detected by qPCR assay was highest on day 1 after EHV-1 inoculation, 2 of 6 horses that received placebo, 2 of 6 horses that received valacyclovir treatment at detection of fever, and 5 of 6 horses that received valacyclovir treatment prior to viral challenge had higher viral shedding on day 2 than on 1 day after EHV-1 inoculation. By 2 days after EHV-1 inoculation, horses in the treatment groups had less shedding of infectious virus than did control horses. Overall, drug treatment significantly reduced nasal shedding of infectious virus during the first few days of treatment ($P = 0.03$ for group by day interaction). Compared with control group findings, valacyclovir administration significantly reduced amounts of viral DNA in nasal swabs (as determined by qPCR assay) throughout the 2-week sample collection period for horses in the prophylactic treatment groups that received valacyclovir for 1 or 2 weeks and horses in the febrile treatment group that received valacyclovir for 2 weeks ($P = 0.001$, 0.0008, and 0.001, respectively). Viral shedding, as determined by qPCR assay, for horses in the febrile treatment group that received valacyclovir for 1 week did not differ ($P = 0.33$) from that of the control horses.

Viral DNA was first detected in the PBMCs of control horses at day 2 after EHV-1 inoculation and reached maximal loads at approximately 5 to 7 days after EHV-1 inoculation (Figure 3). Drug treatment reduced viremia, compared with findings for control horses ($P < 0.001$ for group by day interaction). On days 5 and 6 after EHV-1 inoculation, viremia in each drug treatment group was less than that in the control group. On days 7 through 10 after EHV-1 inoculation, viremia was less than that in the control group for only horses in the prophylactic treatment group that received valacyclovir for 2 weeks and horses in the febrile treatment group that received valacyclovir for 1 week. On day 10, viremia in the horses in the febrile treatment group that received valacyclovir for 2 weeks was again less than that in the control group, but there was no difference thereafter. Among the tissues collected at necropsy, EHV-1 was most commonly detected by qPCR assay in the nasal turbinates, followed by the lymph nodes and brain (Table 1). Viral load in the CSF samples was highly variable and did not differ among groups ($P = 0.8$). Horses in both prophylactic treatment groups and horses in the febrile treatment group that received valacyclovir for 1

week had lower ($P = 0.02$) total organ viral loads, compared with horses in the control group.

Clinical signs

The control horses had a biphasic fever response, all becoming initially febrile (rectal temperature ≥ 38.5°C) by 1 to 3 days after EHV-1 inoculation and febrile again at 4 to 7 days after EHV-1 inoculation (Figure 4). Treatment group and day after inoculation significantly affected ($P < 0.001$) rectal temperature; however, only horses in the prophylactic treatment group that received valacyclovir for 2 weeks had rectal temperatures that were significantly ($P < 0.001$) lower by the multiple comparisons procedure than those of control horses over the course of the study. All 6 horses in the 2 febrile treatment groups became febrile by 2 days after EHV-1 inoculation; valacyclovir treatment was initiated on the first day after EHV-1 inoculation in 4 horses and on the second day after EHV-1 inoculation in 2 horses. Although the study was not designed to assess differences in the effects of beginning treatment on day 1 versus day 2 after EHV-1 inoculation, there were no obvious differences in clinical signs or other outcomes between horses that had valacyclovir administration initiated 1 or 2 days after EHV-1 inoculation. Data for all the horses in the febrile treatment groups that were retained in the final analysis were indicative of a second febrile response beginning 3 to 7 days after EHV-1 inoculation. Two horses in the prophylactic treatment groups failed to develop fever, whereas the other 4 horses first became febrile between 1 and 3 days after EHV-1 inoculation. Of these, 3 horses had a second febrile episode at 7 or 8 days after EHV-1 inoculation.

Daily clinical scores increased rapidly after viral inoculation and continued to increase in control horses until approximately 6 days after EHV-1 inoculation, when scores plateaued but remained high (compared with baseline scores) for the duration of the study. The mean daily clinical score (derived from data obtained on days 2 through 14 after EHV-1 inoculation) was lower ($P = 0.02$) in the horses in the prophylactic treatment group that received valacyclovir for 2 weeks after viral inoculation, compared with that for control horses. The mean daily clinical score for horses in the prophylactic treatment group that received valacyclovir for 1 week after viral inoculation and horses in the febrile treatment group that received valacyclovir for 1 or 2 weeks after viral inoculation did not differ from control group findings ($P = 0.09$, 0.32, 0.99, respectively; Table 2). Mean daily clinical scores for horses treated for 2 weeks were lower than those for horses treated for 1 week ($P = 0.03$).

Ataxia in 2 control horses was severe and progressive (Table 3), requiring euthanasia on days 11 to 14 days after EHV-1 inoculation, whereas no valacyclovir-treated horses required euthanasia. Ataxia was noted as early as 4 days after EHV-1 inoculation, but median ataxia scores in control horses did not increase until 8 days after EHV-1 inoculation (Figure 5). Ataxia was evident in all groups, and horses in the prophylactic treatment and febrile treatment groups had a similar severity of ataxia. Therefore, data for those treatment groups were combined to assess the effect of valacyclovir treatment on the severity of ataxia in EHV-1-inoculated horses, regardless of the day on which treatment was initiated. Drug administration significantly reduced the severity of ataxia in EHV-1-inoculated horses;

compared with findings for control horses, the proportion of valacyclovir-treated horses that had a change in ataxia score of ≥ 2 grades was significantly ($P = 0.02$) smaller.

Safety and pharmacokinetic and pharmacodynamic parameters of valacyclovir administration

In the study horses, several CBC and plasma biochemical variables, such as cholesterol concentration, were just outside the reference range; however, these abnormalities were consistent both before and after valacyclovir administration (Table 4). Overall, no clinically important abnormalities attributable to drug administration were revealed by the CBC, plasma biochemical analysis, or microscopic examination of CSF samples and bone marrow aspirates performed for any horse that had received 2 weeks of valacyclovir treatment after viral inoculation.

A 2-compartment model with first-order absorption best described the plasma acyclovir concentration data obtained subsequent to oral administration of valacyclovir (Figure 6). Trough plasma acyclovir concentrations were approximately 40 times the time-matched CSF acyclovir concentrations (Table 5). Trough acyclovir plasma concentration was the pharmacokinetic parameter that was best correlated with reduction of viral load in PBMCs; thus, trough acyclovir plasma concentrations were used to calculate the in vivo IC_{50} (Figure 7). The in vitro mean ± SE IC_{50} of acyclovir was 11.4 ± 1.5 μg/mL; that of penciclovir and ganciclovir was 4.8 ± 0.7 μg/mL and 0.1 ± 0.1 μg/mL, respectively. In contrast, the in vivo IC_{50} of plasma acyclovir was 0.89 μg/mL. When stepwise multiple linear regression analysis was used to determine which pharmacokinetic parameters were predictive of EHV-1 viremia in PBMCs, $C_{min(ss)}$ and percentage of the dosing interval in which plasma acyclovir concentration exceeded the in vivo IC_{50} (% $\tau > IC_{50(\text{in vivo})}$) were significantly ($P < 0.05$) associated with viremia, whereas $C_{max(ss)}$ and $AUC_{\tau(ss)}$ were not significant factors. The addition of % $\tau > IC_{50(\text{in vivo})}$ did not significantly improve the model, compared with use of $C_{min(ss)}$ alone.

Discussion

Adverse effects reported following administration of high doses (240 mg/kg/d) of valacyclovir in species other than horses have centered on the hematopoietic, renal, and hepatic systems.[22] Because there were no changes in physical examination findings, clinicopathologic variables, or the microscopic appearance of CSF samples or bone marrow aspirates attributable to valacyclovir administration for 2 weeks after viral inoculation in the horses of the present study, the dosing regimen used appeared to be safe in horses.

The administration of valacyclovir to horses within 2 days after viral inoculation had a positive effect on several key outcomes after EHV-1 inoculation, including viral shedding, viremia, pyrexia, clinical score, and severity of ataxia. The mean values for nasal shedding of EHV-1 were highest on day 1 after inoculation. We were not able to determine whether the shed virus was the product of nasal replication or a residual fraction of the inoculum, but given that multiple horses developed clinical signs of infection (eg, fever), nasal replication seemed to be the most likely explanation. Nasal shedding of EHV-1 was determined in nasal swab samples by both virus isolation and qPCR assay. Viral isolation was performed in a

semiquantitative manner, as PFU were not counted directly from the nasal swab samples; swab-borne virus was first inoculated onto cultured cells before being titrated onto additional wells for counting. Although the absolute count of PFU from this method is inaccurate, this method allows for the estimation of the temporal pattern of viral shedding and relative amounts of replicative virus over time in the different groups of study horses and is a technique that we have successfully used in the past.[23] However, qPCR assay was used in the present study to assess the actual quantity of virus from nasal swabs. Over the 14-day study period following viral inoculation, nasal shedding of EHV-1 in horses that received valacyclovir starting the day before viral challenge and continuing for 1 to 2 weeks following viral inoculation or starting at the detection of fever and continuing for 2 weeks was lower than that of control horses.

Viremia was also greatly reduced in valacyclovir-treated horses, compared with control horses, with approximately 1,000-fold lower maximal viral load in PBMCs at 4 to 5 days after EHV-1 inoculation and 45-fold lower viral loads at 6 to 7 days after EHV-1 inoculation in the treated horses. Among the valacyclovir-treated horses, the magnitude of viremia depended on $C_{min(ss)}$. Any intervention that reduces viremia is expected to reduce the likelihood of EHV-1-related neurologic disease development because the magnitude of viremia has been implicated in the development of EHM.[1] In the present study, 10- to 1,000-fold decreases in viral loads of EHV-1 in various tissues were also detected in nearly all tissues from valacy-clovir-treated horses, compared with viral loads in similar tissues from control horses, supporting the hypothesis that viremia directly affects viral load in tissues.

In addition to quantitatively decreasing viral replication, valacyclovir treatment also affected clinical variables. As expected, the most aggressive tested valacyclovir dosing regimen, where treatment was started prior to viral challenge and continued for 2 weeks, was more effective at minimizing pyrexia than was treatment that began only after horses became febrile or was discontinued after only 1 week. With regard to mean daily clinical scores, only the value for horses in the prophylactic treatment group that received valacyclovir for 2 weeks after EHV-1 inoculation was significantly less than that of the horses in the control group. The greatest differences in clinical scores between those 2 groups were reductions in respiratory rate, heart rate, and nasal discharge. Overall, however, there were few differences in the measured outcomes that could be attributed to the duration of valacyclovir administration (1 week vs 2 weeks after viral inoculation). The timing of the initiation of valacyclovir treatment did not appear to affect neurologic outcomes, which were similar for horses in the 4 antiviral treatment groups. When all valacyclovir treatment groups were considered together, valacyclovir administration did not significantly reduce the incidence of ataxia, but did decrease the severity of ataxia. Only 1 of 11 valacyclovir-treated horses, for which valacyclovir treatment was initiated 1 day before EHV-1 inoculation and continued for 1 week, developed a 2-grade change in ataxia score, whereas 4 of 6 control horses developed a 2-grade change in ataxia score.

The absorption profile of multiple doses of valacyclovir administered orally to horses was similar to that previously determined in a single-dose study.[7] As predicted, the loading dose regimen allowed plasma acyclovir concentrations to rapidly reach steady-state concentrations. However, trough plasma concentrations were approximately half of

predicted values. This difference could primarily be attributed to the shorter-than-predicted elimination half-life ($t_{1/2(\beta)}$), which was also highly variable. Although data from single-dose pharmacokinetic studies can be used to make predictions about multiple-dose pharmacokinetic parameters, actual multiple-dose kinetics may differ from such predictions, as appeared to be the case in the study reported here. Horses with longer elimination half-life had correspondingly higher trough acyclovir concentration. The in vitro IC_{50} for acyclovir, determined in the present study by use of the same T953 strain of EHV-1 used to inoculate the horses, was approximately 10 times the suppressive acyclovir concentrations determined from the live animal data. Our estimated in vitro IC_{50} of acyclovir was 11.4 μg/mL, which was also higher than values (0.45 to 7 μg/mL) determined in vitro for 10 neuropathogenic and abortive isolates of EHV.[8,10,24–26] However, our estimated IC_{50} values for penciclovir (4.8 μg/mL) and ganciclovir (0.1 μg/mL) for the same T953 strain were similar to reported values for other EHV-1 isolates.[8,10,24,25,27–29] Other investigators have also reported that in vitro drug efficacy does not always predict in vivo drug efficacy.[28,30] The basis of this discrepancy in the present study was unclear, but the discrepancy highlights the importance of in vivo data when examining the efficacy of antiviral drugs. Valacyclovir treatment decreased viremia in the horses of the present study when trough plasma concentrations were maintained > 0.8 μg/mL. Furthermore, $C_{min(ss)}$ and percentage of the dosing interval during which plasma acyclovir concentrations exceeded 0.8 μg/ mL were best correlated with a reduction in viremia. Therefore, acyclovir appears to follow a time-dependent pharmacokinetic-pharmacodynamic pattern, consistent with its virostatic mechanism of action.[31]

One of the horses for which valacyclovir treatment was initiated 1 day prior to EHV-1 inoculation and continued for 1 week after inoculation had the highest level of viremia, lowest trough acyclovir plasma concentration, most severe neurologic signs, and most pronounced histologic lesions in the spinal cord (vasculitis consistent with herpesvirus infection located in the cervical intumescence), as compared with its treated cohorts. To maintain trough plasma acyclovir concentration near 0.8 μg/mL in this horse, pharmacokinetic simulations indicated that it would have been necessary to administer valacyclovir at a maintenance rate of 36 mg/kg, PO, every 12 hours. However, a maintenance dosage of 30 mg/kg, PO, every 12 hours would be expected to maintain acyclovir concentrations > 1 μg/mL for at least half of the dosing interval, consistent with the reported antiviral efficacy for acyclovir.[7] Dosages of valacyclovir may need to be increased beyond those used in the present study to obtain more uniform efficacy. One practical suggestion would be continuation of the loading dose regimen in horses that respond poorly to treatment, such as horses that continue to be febrile.

In the present study, both laboratory and clinical data supported the efficacy of early valacyclovir treatment in protecting horses from the most severe sequelae of EHV-1 infection. The efficacy of valacyclovir treatment in a susceptible population of horses was in contrast to a previous report of therapeutic failure of valacyclovir after administration at a dosage of 40 mg/kg, PO, every 8 hours for 5 to 7 days in 4 weanling pony foals inoculated with EHV-1.[32] There were several differences between that and the present study that could explain this discrepancy, such as differences in disease susceptibilities between weanling foals and aged horses and between ponies and horses, strain of EHV-1 used for inoculation,

duration of treatment, and sample size. The clinical relevance of the present study was reflected in protection of horses from the major clinical outcome of interest, the development of EHM, which may occur in < 10% of experimentally inoculated young horses.[1] Because EHM develops so rarely in younger horses after experimental inoculation with EHV-1, we selected a population of horses (geriatric mares) with a previously reported enhanced susceptibility to development of EHM.[1,33] The use of aged mares may allow the assessment of the efficacy of interventions that may protect horses from the devastating neurologic effects of EHV-1 infection in controlled, experimental settings.

Although oral valacyclovir treatment did not ablate infection with neuropathogenic EHV-1 in most horses in the present study, it did significantly decrease viral replication, viral dissemination, and severity of clinical signs of disease. The protective effect of valacyclovir treatment was greatest when treatment was initiated before viral inoculation and continued for 2 weeks after inoculation. However, valacyclovir treatment also decreased clinical signs when treatment initiation was delayed by as long as 2 days after EHV-1 inoculation. During an outbreak of EHM, antiviral treatment may be initiated in horses at various stages of infection, including horses that have not yet developed clinical signs of viral disease.

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Appendix 1

Modified Mayhew ataxia severity scale used to assess horses before and after inoculation with EHV-1 and treatment with placebo or valacyclovir.

Score
0 = Normal; neurologic deficits not detected.
1 = Neurologic deficit may be detectable at normal gaits; exacerbated with manipulative procedures.
2 = Neurologic deficit obvious at normal gaits or posture; signs exacerbated with manipulative procedures.
3 = Neurologic deficit very prominent at normal gaits; horse gives the impression that it may fall (but does not) and buckle or fall with manipulative procedures.
4 = Neurologic deficit is profound at normal gait; horse frequently stumbles or trips and may fall at normal gaits or when manipulative procedures are applied.
5 = Horse is recumbent and unable to rise.

Appendix 2

Scoring system for clinical signs of infection in horses inoculated with EHV-1 and treated with placebo or valacyclovir.

Clinical sign	Score
Appetite	0= Horse immediately eats the concentrates offered 1= Poorly interested in feed, but still comes to feed bucket after delay of 1 min 2= Not interested in feed
Attitude	0 = Normal, bright, alert, and responsive 1 = Quiet, but alert and responsive when stimulated 2 = Mentally dull and lethargic 3 = Signs of severe depression, and unable to stand without assistance
Respiratory rate	0 = Normal respiratory rate (≤ 20 breaths/min) 1 = Mildly increased (21–30 breaths/min) 2 = Moderately increased (31–40 breaths/min) 3 = Severely increased (> 40 breaths/min)
Heart rate	0 = Normal heart rate (≤ 40 beats/min) 1 = Mildly increased (41–48 beats/min) 2 = Moderately increased (49–59 beats/min) 3 = Severely increased (> 59 beats/min)
Serous nasal discharge	0 = No discharge 1 = Slight amount of discharge 2 = Moderate amount of discharge 3 = Severe amount of discharge
Mucopurulent nasal discharge	0 = No discharge 1.5 = Some discharge 3.0 = Moderate amount of discharge 4.5 = Severe amount of discharge
Serous ocular discharge	0 = No discharge 1 = Slight amount of discharge 2 = Moderate amount of discharge 3 = Severe amount of discharge
Mucopurulent ocular discharge	0 = No discharge 1.5 = Slight amount of discharge 3.0 = Moderate amount of discharge 4.5 = Severe amount of discharge
Mandibular lymph node	0 = Barely palpable 1 = Cherry-sized (approx 15 × 10 × 10 mm) 2 = Walnut-sized (approx 30 × 20 × 20 mm) 3 = Mandarin orange–sized (approx 50 × 25 × 25 mm)

ABBREVIATIONS

$AUC_{\tau(ss)}$	Area under the plasma acyclovir concentration versus time curve during a dosing interval at steady state
$C_{max(ss)}$	Maximal plasma acyclovir concentration at steady state
$C_{min(ss)}$	Trough plasma acyclovir concentration at steady state
EHM	Equine herpesvirus myeloencephalopathy
EHV-1	Equine herpesvirus type-1
IC_{50}	Concentration of acyclovir required to inhibit plaque formation by 50%
PBMC	Peripheral blood mononuclear cell

PFU	Plaque-forming units
qPCR	Quantitative PCR

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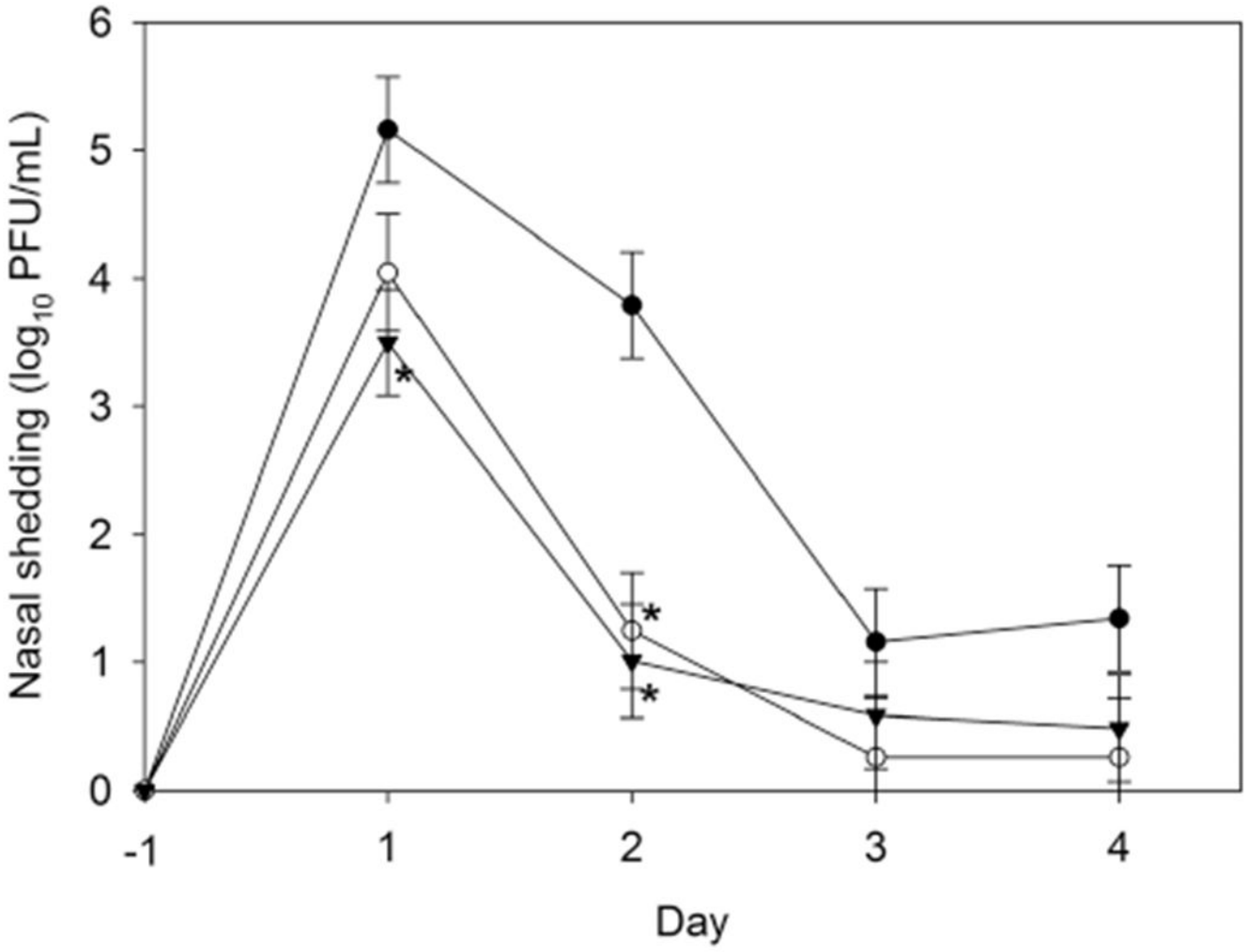

Figure 1—.

Mean ± SEM shedding of EHV-1 as determined by virus isolation from nasal swab samples collected from horses that received an oral placebo (control; black circles; n = 6), valacyclovir hydrochloride treatment at detection of fever (ie, rectal temperature ≥ 38.5°C [febrile treatment group; white circles; 5]), or prophylactic valacyclovir hydrochloride treatment (initiated 1 day prior to viral challenge [prophylactic treatment group; inverted triangles; 6]) and were inoculated intranasally with a neuropathogenic strain of EHV-1 (10^7 PFU) on day 0. Placebo or valacyclovir was administered orally for 7 or 14 days after EHV-1 inoculation or detection of fever (3 horses/group) in control and valacyclovir-treated horses. The drug was administered at a loading dose rate of 27 mg/kg, PO, every 8 hours for 2 days, followed by a maintenance dose of 18 mg/kg, PO, every 12 hours for the duration of the assigned treatment period. Nasopharyngeal swab samples were collected on the day before EHV-1 inoculation (day -1) and once daily thereafter until 14 days after inoculation. Virus was isolated as late as 9 days after EHV-1 inoculation, but because viral isolation was achieved in only 2 horses on day 5 and in 1 horse on days 6 to 9 after EHV-1 inoculation, statistical comparisons were restricted to data obtained on the first 4 days after viral challenge. Data for 1 horse in the febrile treatment group that received valacyclovir for 2

weeks after viral inoculation were not included because the horse had preexisting bronchopneumonia. *At this time point, value is significantly ($P < 0.05$) different from that for the control horses.

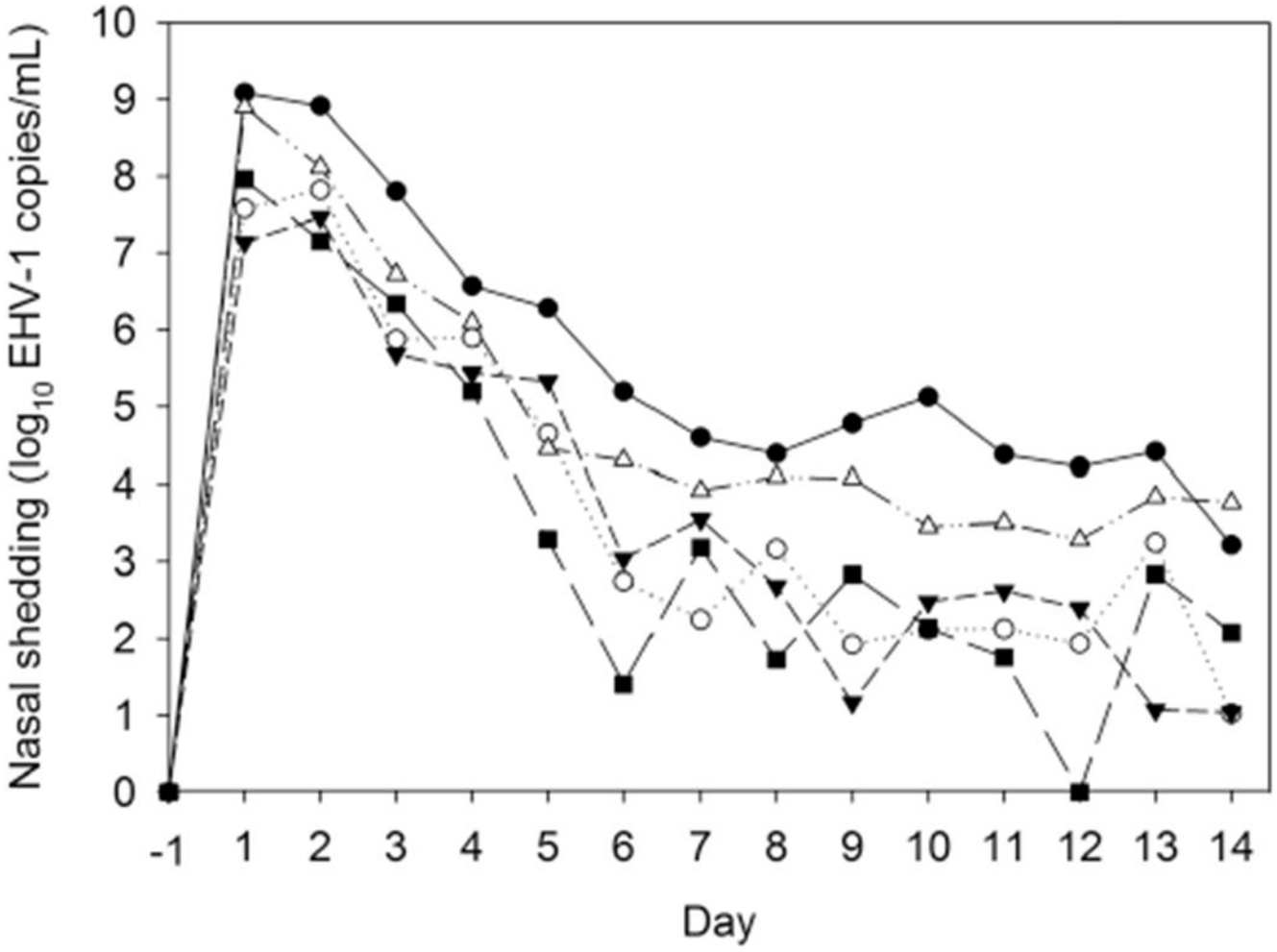

Figure 2—.

Mean ± SEM shedding of EHV-1 as determined by qPCR assay in nasal swab samples collected from horses in the control group that received an oral placebo for 1 or 2 weeks (combined data; black circles; n = 6), horses in the febrile treatment group that received valacyclovir for 1 week (white triangles; 3), horses in the febrile treatment group that received valacyclovir for 2 weeks (squares; 2), horses in the prophylactic treatment group that received valacyclovir for 1 week (white circles; 3), or horses in the prophylactic treatment group that received valacyclovir for 2 weeks (inverted black triangles; 3) and were inoculated with EHV-1 on day 0. Data for 1 horse in the febrile treatment group that received valacyclovir for 2 weeks after viral inoculation were not included because the horse had preexisting bronchopneumonia. One horse in the control group was euthanized on day 11; there were no data from this horse on days 12, 13, and 14. A second horse in the control group was euthanized on day 14; there were no data from this horse on day 14. Values were significantly lower after viral inoculation in horses that received valacyclovir prophylactically for 1 or 2 weeks or in the febrile treatment group that received valacyclovir for 2 weeks (P = 0.001,0.0008, and 0.001, respectively), compared with control horses. ***See*** Figure 1 for remainder of key.

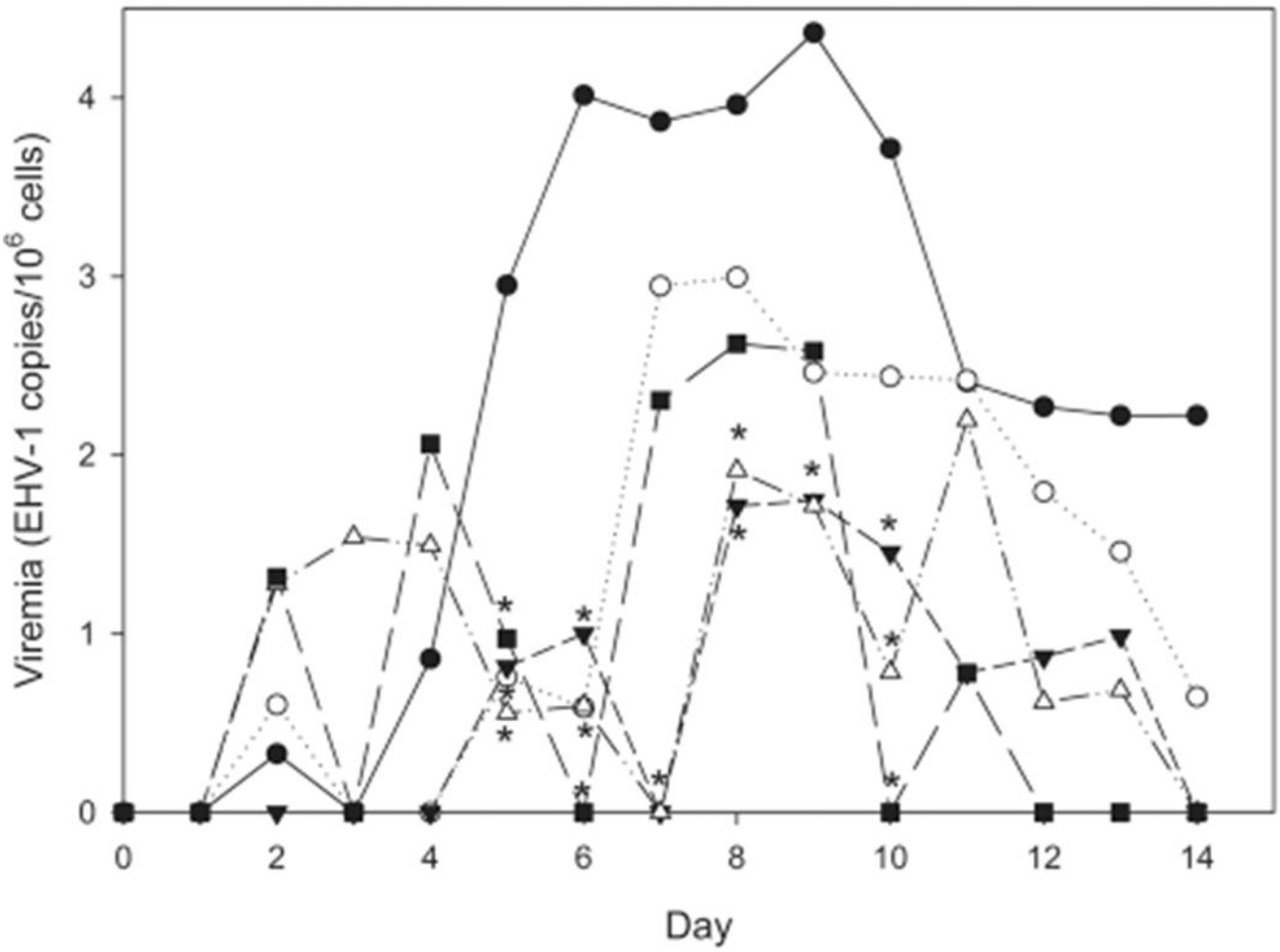

Figure 3—.

Viremia (determined on the basis of amounts of viral DNA detected in PBMCs) in horses in the control group, horses in the 2 prophylactic treatment groups, and horses in the 2 febrile treatment groups for which valacyclovir treatment was commenced within 2 days after EHV-1 inoculation (at detection of fever). From each horse, blood samples were collected for isolation of PBMCs before inoculation with EHV-1 on day 0 and every day through 14 days after viral inoculation. Viremia was quantified by a qPCR assay. Data are reported as mean ± SEM. ***See*** Figures 1 and 2 for remainder of key.

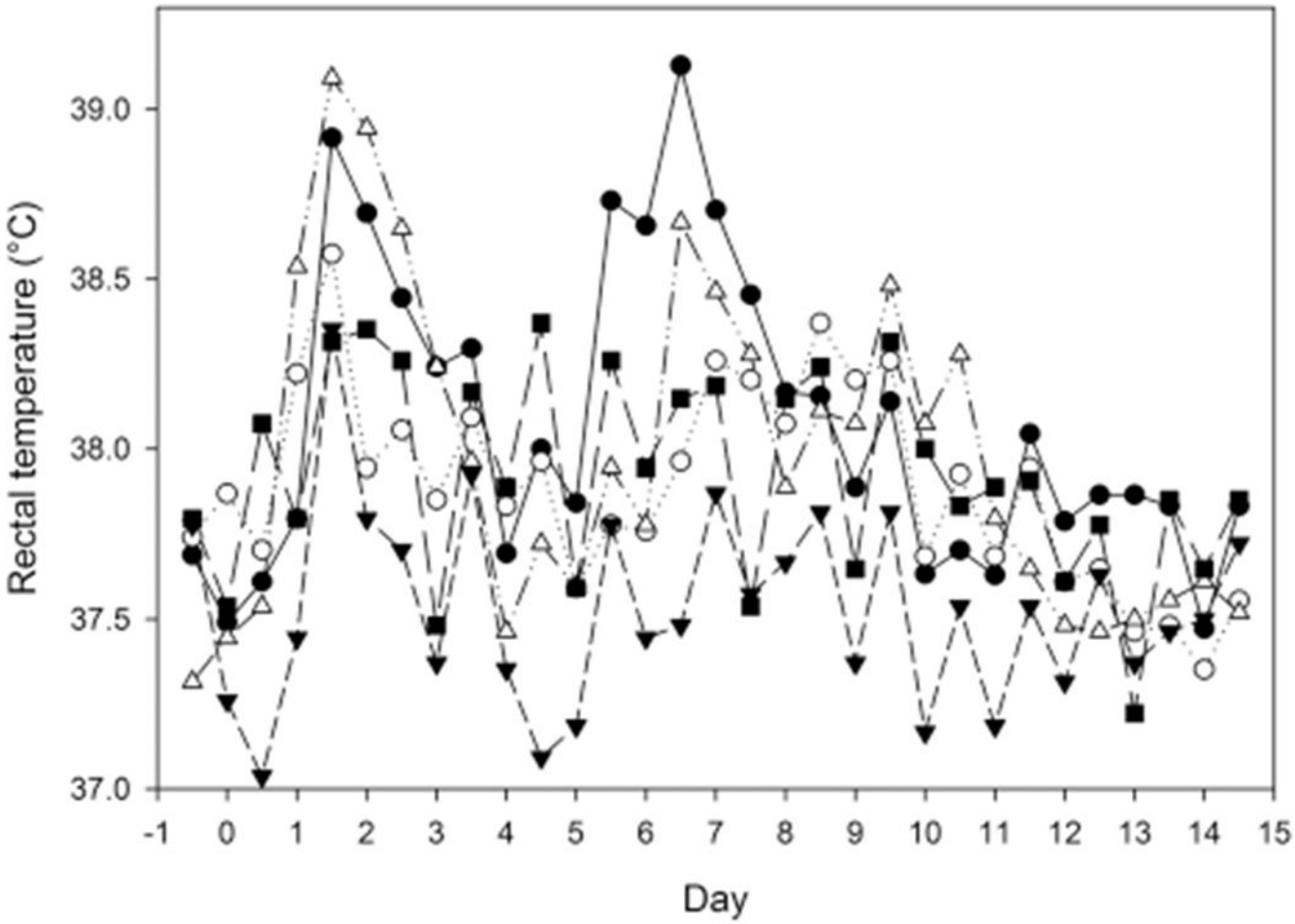

Figure 4—.

Mean rectal temperature in horses in the control group, horses in the 2 prophylactic treatment groups, and horses in the 2 febrile treatment groups for which valacyclovir treatment was commenced within 2 days after EHV-1 inoculation (at detection of fever). For all horses, rectal temperature was determined twice daily from days -2 through 14 after EHV-1 inoculation, and each measurement was used as a separate data point. However, statistical analysis was performed on data from -0.5 days onward because earlier time points were used to train horses to the procedure and to confirm that horses were healthy. Values were significantly ($P < 0.001$) lower after viral inoculation in horses that received valacyclovir prophylactically for 2 weeks, compared with values in control horses. ***See*** Figures 1 and 2 for remainder of key.

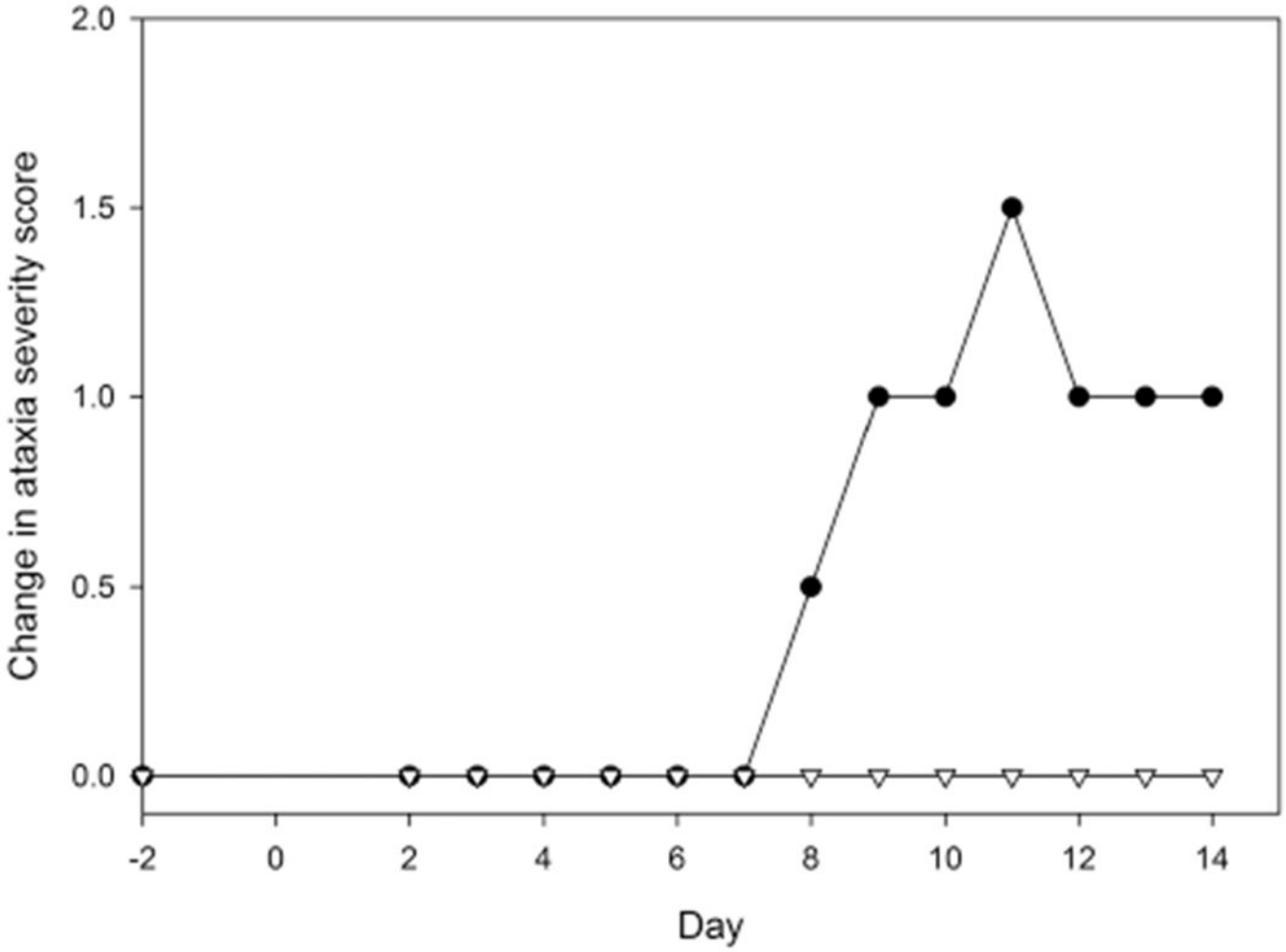

Figure 5—.

Median change in ataxia severity score in horses in the control groups that received placebo for 1 or 2 weeks (circles; n = 6), horses in the prophylactic treatment groups that received valacyclovir for 1 or 2 weeks (6), and horses in the febrile treatment groups that received valacyclovir for 1 or 2 weeks (5) after EHV-1 inoculation. Neurologic examinations were performed once daily at day -2 and from days 2 through 14. Ataxia was graded on a 6-point ataxia severity scale; statistical comparisons were not performed on the day-by-day ataxia scores. Ataxia scores from the control horses that were euthanized for ataxia on days 11 and 14 were included on those days. Horses that received any valacyclovir regimen are represented as inverted triangles. ***See*** Figures 1 and 2 for remainder of key.

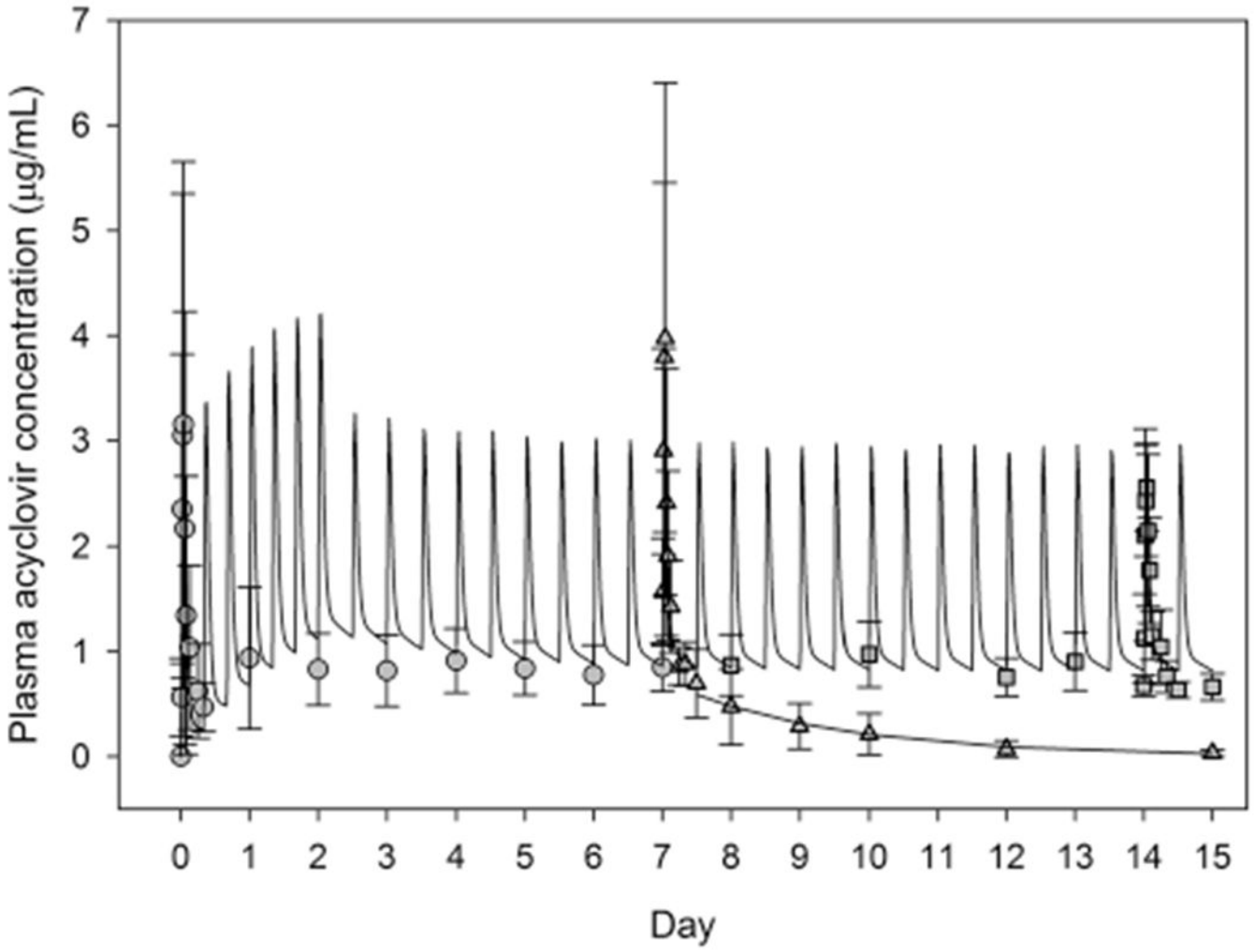

Figure 6—.

Mean ± SD plasma acyclovir concentrations in horses that received valacyclovir treatment for 1 week (triangles; n = 6) or 2 weeks (squares; 6) after EHV-1 inoculation on day 0 and all valacyclovir-treated horses (circles; 12). Drug treatment was initiated prophylactically (initiated 1 day prior to viral challenge; n = 6) or at detection of fever (on days 1 or 2; 6). The drug was administered at a loading dosage of 27 mg/kg, PO, every 8 hours for 2 days, followed by a maintenance dosage of 18 mg/kg, PO, every 12 hours for the duration of the assigned treatment period. Samples of blood were collected at 0 (immediately before drug administration), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 8, and 12 hours after the administration of valacyclovir on the first and seventh days after EHV-1 inoculation in all valacyclovir-treated horses and additionally at those time points 14 days after inoculation in the horses that received 2 weeks of treatment. To determine trough plasma valacyclovir concentrations, blood samples (10 mL each) were collected in the morning, just before the next dose of valacyclovir, on days 2 through 6 from all valacyclovir-treated horses and additionally on days 8, 10, 12, 13, 14, and 15. On day 15 after EHV-1 inoculation, the trough blood sample was obtained immediately before euthanasia by IV overdose injection of pentobarbital. Solid

lines represent the predicted plasma acyclovir concentrations. ***See*** Figure 1 for remainder of key.

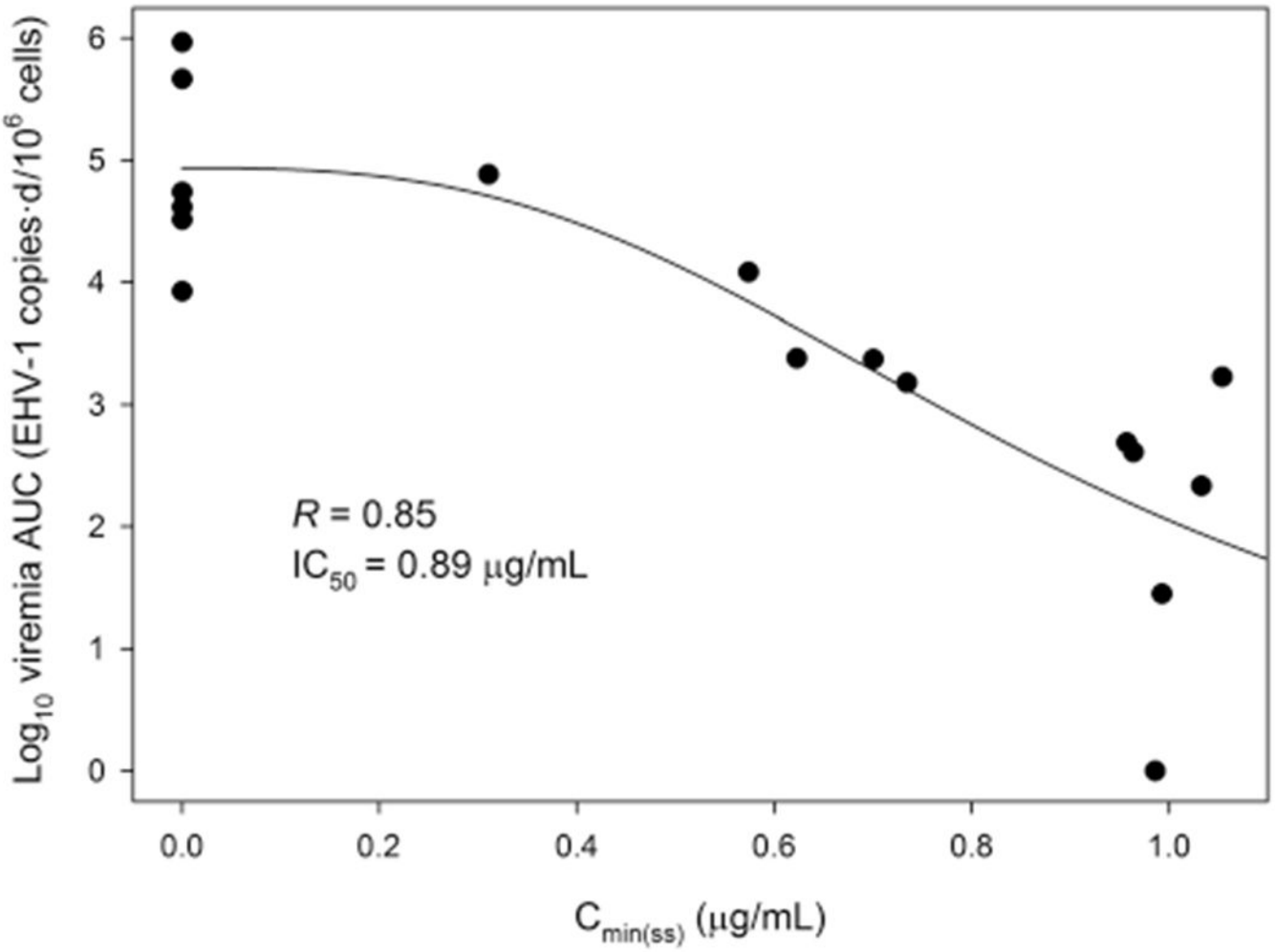

Figure 7—.

In vivo IC_{50} from the sigmoidal inhibitory E_{max} model relating viremia (expressed as log_{10} AUC of EHV-1 copies•d/10^6 cells) and $C_{min(ss)}$. All horses (n = 17), both control and valacyclovir-treated horses, were included in this analysis, with the exclusion of data for 1 horse in the febrile treatment group that received valacyclovir for 2 weeks after viral inoculation that were not included because the horse had preexisting bronchopneumonia that may have affected viral disease progression. ***See*** Figure 1 for remainder of key.

Table 1—

Mean ± SEM $\log_{10}$ viral load (EHV-1 copies/10^6 cells) in CSF and tissue samples obtained from horses that were inoculated intranasally with a neuropathogenic strain of EHV-1 (10^7 PFU) on day 0 and received a placebo (control; initiated on day 0), valacyclovir hydrochloride treatment at detection of fever (febrile treatment group), or prophylactic valacyclovir hydrochloride treatment (initiated 1 day prior to viral challenge [prophylactic treatment group]) orally for 7 or 14 days.

Sample	Treatment duration after EHV-1 inoculation (wk)	Control group	Febrile treatment group	Prophylactic treatment group
CSF	1	5.7 ± 0.1	2.0 ± 2.0	1.7 ± 1.7
	2	2.5 ± 2.5	0	2.0 ± 2.0
Outer cerebral cortex	1	2.8 ± 1.5	0.8 ± 0.8	0.8 ± 0.8
	2	2.1 ± 1.3	0	0
Gray matter of cerebrum	1	1.5 ± 1.5	0.7 ± 0.7	0.9 ± 0.9
	2	2.5 ± 1.5	0	0
White matter of cerebrum	1	1 ± 1	0	0
	2	0	0	0
Cervical spinal cord	1	0	0	0.9 ± 0.9
	2	0	0	0
Thoracic spinal cord	1	2.2 ± 1.3	0	0
	2	1.3 ± 1.3	0	0
Lumbar spinal cord	1	0	0	0
	2	0.9 ± 0.9	0	0
Nasal turbinates	1	2.3 ± 1.1	0.5 ± 0.5	1.0 ± 0.5
	2	4.8 ± 0.8	0	0
Mandibular lymph nodes	1	3.2 ± 0.5	0	0
	2	3.2 ± 0.6	2.1 ± 0.5	0.9 ± 0.9
Auditory tube diverticula (guttural pouches)	1	1.2 ± 1.2	0	0
	2	2.5 ± 0.3	0	0
Pharynx	1	1.4 ± 1.4	0	0
	2	1.2 ± 0.6	0	0
Lungs	1	1.6 ± 0.9	0	0
	2	1.4 ± 0.8	0	0
Liver	1	0.9 ± 0.9	0	0
	2	1.6 ± 0.8	0	0
Kidneys	1	1.3 ± 1.3	0	0
	2	2.6 ± 1.5	0	1.2 ± 1.2
All tissues	1	4.2 ± 0.8	1.5 * ± 0.8	1.9 * ± 0.9
	2	5.5 ± 0.3	2.1 ± 0.5	1.2 * ± 1.2

Horses in the control group received an oral placebo (flour mixed with corn syrup) daily for 7 or 14 days after EHV-1 inoculation. For horses in the febrile treatment group, valacyclovir treatment was initiated at the time of detection of fever (ie, rectal temperature ≥ 38.5°C); for horses in the prophylactic treatment group, valacyclovir treatment was initiated 1 day prior to viral challenge. In each treatment

group, valacyclovir was ad ministered orally for 7 or 14 days after EHV-1 inoculation or detection of fever (3 horses/group). The drug was administered at a loading dose rate of 27 mg/kg, PO, every 8 hours for 2 days, followed by a maintenance dose of 18 mg/kg, PO, every 12 hours for the duration of the assigned treatment period. Each treated and control horse was euthanized and necropsied on day 15 after inoculation, except for 2 horses (control group) that were euthanized and necropsied on days 11 and 14 because of progression of neurologic disease. Data for 1 horse in the febrile treatment group that received valacyclovir for 2 weeks after viral inoculation were not included because the horse had preexisting bronchopneumonia. A CSF sample was collected from the atlanto-occipital space, and fresh tissue samples were collected. The viral load in samples of each tissue was quantified by qPCR assay.

*For a given treatment duration, value is significantly ($P = 0.02$) different from that of the control group.

Table 2—

Median values of the mean daily clinical score for the horses in Table 1 on days 2 through 14 after EHV-1 inoculation on day 0.

Clinical sign	Treatment duration after EHV-1 inoculation (wk)	Control group	Febrile treatment group	Prophylactic treatment group
Appetite	1	0	0.2	0
	2	0	0.2	0
Attitude	1	0.6	0.4	0.6
	2	0.5	0.8	0.1
Respiratory rate	1	0.5	1.0	0.2
	2	1.2	1.5	0.4
Heart rate	1	0.5	0	0.2
	2	0.5	0.6	0.0
Nasal discharge	1	0.6	0.8	0.3
	2	1.2	0.6	0.4
Ocular discharge	1	0.5	1.0	0.2
	2	0.2	0.6	0.4
Mandibular or retropharyngeal lymph node	1	1.9	0.5	2.2
	2	1.5	1.1	1.1
All clinical signs (cumulative clinical score)	1	4.2	4.3	3.2
	2	4.6	5.4	2.0†

Horses were examined once daily. A score for each clinical sign was assigned and a daily clinical score for each horse was generated by summing these clinical signs, an adaptation of a previously reported method.[16] The mean daily clinical score for each horse was the sum of its daily clinical scores from day 2 through day 14 divided by the number of days that the horse remained in the study during that time period. The maximum daily clinical score for any given horse was 23.

†For a given treatment duration, value was significantly ($P = 0.02$) different from that of the control group.

See Table 1 for remainder of key.

Table 3—

Proportions of control and valacyclovir-treated horses in Table 1 that had a change from baseline ataxia severity score (day –2), as determined by 2 observers.

Variable	Treatment duration after EHV-1 inoculation (wk)	Control group	Febrile treatment group	Prophylactic treatment group	All valacyclovir-treated horses
Change in ataxia of ≥ 1 grade	1	2/3	1/3	1/3	—
	2	2/3	1/2	0/3	—
	Combined[a]	4/6	—	—	3/11
Change in ataxia of ≥ 2 grades	1	2/3	0/3	1/3	—
	2	2/3	0/2	0/3	—
	Combined[a]	4/6	—	—	1/11[≠]
Horse became recumbent	1	2/3	0/3	0/3	—
	2	0/3	0/2	0/3	—
	Combined[a]	2/6	—	—	0/11

Neurologic examinations were performed daily by a masked board-certified equine internist at day –2 and on days 2 through 14 after EHV-1 inoculation. Neurologic examinations were also recorded on video, digitized, randomized with respect to horse and time, and then evaluated by a second board-certified equine internist who was also unaware of the horse's group assignment. Ataxia was graded on a modified 6-point (0 to 5) ataxia scale.

[a]For statistical comparisons, the proportion of control horses (n = 6) was compared with the proportion of all horses that received valacyclo vir treatment (11).

[≠]For this variable, the proportion of all valacyclovir-treated horses differed significantly ($P < 0.05$) from the proportion of the combined control group.

— = Not applicable.

See Table 1 for remainder of key.

Table 4—

Mean ± SEM plasma biochemical and CBC variables determined before and on days 7 and 14 after EHV-1 inoculation (on day 0) for the horses in Table 1 that received placebo (control) or valacyclovir (for 1 or 2 weeks) after inoculation or detection of fever.

		Prior to viral inoculation		Day 7 after viral inoculation		Day 14 after viral inoculation	
Variable	**Reference interval**	**Control (n = 6)**	**Valacyclovir (n = 12)**	**Control (n = 6)**	**Valacyclovir (n = 12)**	**Control (n = 2)**	**Valacyclovir (n = 6)**
Aspartate aminotransferase (U/L)	180–570	229 ± 17	195 ± 23	203 ± 12	200 ± 15	178 ± 23	198 ± 23
Total bilirubin (mg/dL)	0.1–2.5	0.8 ± 0.2	1.4 ± 0.3	1.5 ± 0.2	1.6 ± 0.2	0.6 ± 0.1	1.2 ± 0.1
Alkaline phosphatase (U/L)	50–300	188 ± 14	166 ± 11	155 ± 14	159 ± 20	199 ± 5.0	120 ± 7.0
γ-Glutamyltransferase (U/L)	2–30	5.5 ± 3.3	3.5 ± 0.5	10.2 ± 3.5	9.2 ± 3.2	16.0 ± 0	7.7 ± 2.1
Total protein (g/dL)	5.6–8.0	7.2 ± 0.2	7.3 ± 0.3	7.3 ± 0.2	7.6 ± 0.3	8.2 ± 0.5	8.5 ± 0.3
Albumin (g/dL)	2.2–3.9	2.5 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.6 ± 0.2	2.5 ± 0.1
Globulin (g/dL)	2.6–5.6	4.8 ± 0.3	4.6 ± 0.3	5.0 ± 0.3	5.0 ± 0.3	5.70 ± 0.3	6.0 ± 0.4
Albumin-to-globulin ratio	0.5–2.4	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.15
Cholesterol (mg/dL)	70–150	55 ± 5.0	55 ± 6	56 ± 3.0	51 ± 3.0	68 ± 8.0	51 ± 7.0
BUN (mg/dL)	10–25	19 ± 2.0	15 ± 1.0	13 ± 1.0	13 ± 1.0	15 ± 2.0	14 ± 1.0
Creatinine (mg/dL)	1.2–2.0	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.1
BUN-to-creatinine ratio	5–21	23.8 ± 5.8	18.0 ± 1.3	14.0 ± 1.3	15.2 ± 1.4	18.5 ± 2.5	20.7 ± 2.4
Phosphorus (mg/dL)	2.0–5.6	2.5 ± 0.4	2.8 ± 0.1	2.2 ± 0.2	2.8 ± 0.2	3.8 ± 0.8	3.3 ± 0.3
Calcium (mg/dL)	10.0–13.7	10.7 ± 0.2	11.5 ± 0.2	10.6 ± 0.1	11.4 ± 0.3	10.8 ± 0.1	11.3 ± 0.4
Glucose (mEq/L)	70–120	79 ± 3.0	82 ± 3.0	82 ± 3.0	82 ± 4	113 ± 19	106 ± 4.0
Sodium (mEq/L)	130–146	134 ± 2.0	133 ± 1.0	134 ± 1.0	136 ± 0.6	133 ± 1.0	133 ± 1.0
Potassium (mEq/L)	3–5	3.2 ± 0.2	3.4 ± 0.1	3.7 ± 0.1	3.5 ± 0.1	3.2 ± 0.1	2.9 ± 0.1
Sodium-to-potassium ratio	24–58	43 ± 3.0	39 ± 1.0	36 ± 1.0	39 ± 0.8	41 ± 1.0	46 ± 2.0
Chloride (mEq/L)	95–110	97 ± 1.0	101 ± 1.0	97 ± 1.0	99 ± 0.5	97 ± 2.0	99 ± 1.0
Creatine kinase (U/L)	20–500	295 ± 64	188 ± 23	139 ± 5.0	165 ± 24	158 ± 39	192 ± 18
Lactate dehydrogenase (U/L)	150–450	287 ± 21	252 ± 35	297 ± 12	281 ± 30	294 ± 34	300 ± 42.0
WBC count (× 10^3 cells/μL)	5.5–12.5	9.7 ± 0.9	8.4 ± 0.5	5.7 ± 0.50	7.2 ± 0.6	10.2 ± 1.4	6.5 ± 0.8
RBC count (× 10^6 cells/μL)	6.5–10.5	7.2 ± 0.3	7.8 ± 0.5	6.2 ± 0.2	6.5 ± 0.3	6.4 ± 0.1	5.6 ± 0.1
Hemoglobin (g/dL)	11.0–19.0	12.5 ± 0.4	13.0 ± 0.7	10.8 ± 0.4	10.9 ± 0.4	11.2 ± 0.8	9.5 ± 0.2
Hct (%)	35–52	39.3 ± 1.4	40.9 ± 2.2	34.0 ± 1.4	34.2 ± 1.3	35.5 ± 2.5	30.0 ± 0.5
Mean corpuscular volume (fL)	34–58	54.5 ± 1.0	52.7 ± 0.9	54.3 ± 1.1	52.9 ± 0.8	56.0 ± 3.0	53.5 ± 0.8

		Prior to viral inoculation		Day 7 after viral inoculation		Day 14 after viral inoculation	
Variable	**Reference interval**	**Control (n = 6)**	**Valacyclovir (n = 12)**	**Control (n = 6)**	**Valacyclovir (n = 12)**	**Control (n = 2)**	**Valacyclovir (n = 6)**
Mean corpuscular hemoglobin (pg)	12.3–19.7	17.3 ± 0.4	16.8 ± 0.3	17 ± 0.4	16.8 ± 0.3	17.6 ± 1.0	17.0 ± 0.2
Mean corpuscular hemoglobin concentration(g/dL)	31–37	31.8 ± 0.3	31.8 ± 0.2	32.0 ± 0.0	31.9 ± 0.2	32.0 ± 0.0	31.8 ± 0.2
Neutrophil count (No. of cells/μL)	2,700–6,700	7,168 ± 986	5,701 ± 474	4,039 ± 408	5,424 ± 620	5,986 ± 1090	4,795 ± 808
Lymphocyte count (No. of cells/μL)	1,500–5,500	1,996 ± 362	2,067 ± 260	1,195 ± 193	1,353 ± 185	2,901 ± 231	1,380 ± 92
Monocyte count (No. of cells/μL)	0–800	346 ± 132	274 ± 40	216 ± 54	222 ± 35	557 ± 23	164 ± 47
Eosinophil count (No. of cells/μL)	0–925	362 ± 89	322 ± 62	301 ± 143	171 ± 25	806 ± 6.0	217 ± 36
Basophil count (No. of cells/μL)	0–170	103 ± 84	49 ± 14	48 ± 17	51 ± 38	0.0 ± 0.0	16 ± 16
Platelet count (× 10^3 platelets/μL)	100–400	243 ± 29	289 ± 23	162 ± 8.0	185 ± 8	242 ± 22	209 ± 21
Fibrinogen (mg/dL)	100–400	220 ± 28	277 ± 44	297 ± 7.0	316 ± 35	148 ± 5.0	296 ± 36

Only the 9 horses (3 control and 6 valacyclovir-treated) in the 2-week treatment groups had samples submitted for plasma biochemical and CBC analysis on day 14. Because 1 control horse was euthanized prior to day 14 for progression of neurologic disease, only 2 control horses were available.

See Table 1 for remainder of key.

Table 5—

Mean ± SD pharmacokinetic parameters for valacyclovir in 12 horses in Table 1 that were administered valacyclovir orally at a loading dosage of 27 mg/kg, PO, every 8 hours for 2 days, followed by a maintenance dosage of 18 mg/kg, PO, every 12 hours for 7 or 14 days after EHV-1 inoculation or detection of fever.

Pharmacokinetic parameter	Value
C_{max} (μg/mL)	3.8 ± 2.6
$C_{max(ss)}$ (μg/mL)	3.7 ± 1.9
T_{max} (h)	1.0 ± 0.6
$AUC_{0–8}$	11.4 ± 7.1
$AUC_{\tau(ss)}$	14.3 ± 2.4
A (mg/L)	47 ± 27
B (mg/L)	0.51 ± 0.50
$t_{1/2(abs)}$ (h)	0.29 ± 0.12
$t_{1/2(\alpha)}$ (h)	0.37 ± 0.16
$t_{1/2(\beta)}$ (h)	20.3 ± 22.3
t_{lag} (h)	0.21 ± 0.03
$C_{min(ss)}$ (μg/mL)	0.67 ± 0.14
CSF concentration (μg/mL)	Approx 0.02 ± 0.01
CSF-to-plasma acyclovir concentration ratio (%)	Approx 2.4 ± 0.8

A = Coefficient of distribution. $AUC_{0–8}$ = Area under the plasma drug concentration-time curve from 0 to 8 hours after the first dose. B = Coefficient of elimination. C_{max} = Maximal measured plasma drug concentration after the first dose. CSF concentration = CSF acyclovir concentration at the 12-hour trough of the last dose of valacyclovir. CSF-to-plasma acyclovir concentration ratio = Ratio of CSF acyclovir concentration to time-matched plasma acyclovir concentration, with both obtained at the last 12-hour trough of the final dose of valacyclovir. $t_{1/2(abs)}$ = Absorption half-life. $t_{1/2(\alpha)}$ = Distribution half-life. $t_{1/2(\beta)}$ = Elimination half-life. t_{lag} = Lag time. T_{max} = Time at which C_{max} was detected. ***See*** Table 1 for key.