

STANDARD ARTICLE

Effects of coadministration of corn oil and ponazuril on serum and cerebrospinal fluid concentrations of ponazuril in horses

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Abstract

Background: Ponazuril is used for the treatment of equine protozoal myeloencephalitis (EPM). Coadministration of ponazuril with oil could result in higher serum and cerebrospinal fluid (CSF) concentrations of ponazuril.

Hypothesis: Coadministration of corn oil will result in higher serum and CSF concentrations of ponazuril than when ponazuril is administered alone.

Animals: Ten resident university-owned adult horses of either sex and >2 years of age.

Methods: Cohort study. Ponazuril oral paste (5 mg/kg BW; ponazuril treatment group (PON); n = 5), or ponazuril oral paste (5 mg/kg BW; ponazuril and oil treatment group (PONOIL; n = 5) coadministered with 2 oz of corn oil q24h for 21 days. Horses were treated once daily, for 21 days. Blood was collected on days 0, 7, 14, and 21 before dosing. In addition, CSF was collected on days 1, 7, 14, and 21. The concentration of ponazuril was determined in serum and CSF and results compared using repeated measures ANOVA.

Results: Coadministration of ponazuril with 2 oz of corn oil resulted in higher concentrations of ponazuril in serum (at steady state) than that found in horses given ponazuril alone (6.2 ± 0.9 mg/L versus 4.5 ± 1.0 mg/L; $P = .004$) (mean \pm 1 SD). Cerebrospinal fluid concentrations of ponazuril were also greater in horses that received ponazuril and oil (0.213 mg/L \pm 0.04 versus 0.162 ± 0.04 mg/L) ($P = .03$).

Conclusions and Clinical Importance: Results suggest that coadministration of corn oil with ponazuril might enhance the effectiveness of treatment with ponazuril.

KEYWORDS

anticoagulant, central nervous system, equine protozoal myeloencephalitis, pharmacokinetics

Abbreviations: AO, atlanto-occipital; AQ, albumin quotient; BBB, blood brain barrier; C_{max} , maximal concentration; CSF, cerebrospinal fluid; EPM, equine protozoal myeloencephalitis; HPLC, high-performance liquid chromatography; PON, ponazuril-only treatment group; PONOIL, ponazuril and oil treatment group; RBC, red blood cell; T_{max} , time to maximal concentration.

1 | INTRODUCTION

Ponazuril is intended for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona* with an efficacy of 70% in blinded studies.¹ Treatment failures occur and there is a need to develop strategies to enhance effectiveness of treatment. Absorption

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of ponazuril is rapid after oral administration and steady state concentrations in serum are achieved after 7 days of dosing.² The concentration of ponazuril in the cerebrospinal fluid (CSF) after 7 days of treatment is 0.162 ± 0.05 mg/L (mean \pm 1 SD), and maximal concentrations of ponazuril in the CSF (C_{\max}) are 0.200 ± 0.07 mg/L.² Although the CSF concentration of ponazuril at day 7 exceeds the effective concentration for inhibition of *S. neurona* organisms in vitro,³ the time to maximal concentration (T_{\max}) is 15.4 ± 7.9 days.² This delay in achieving maximal concentrations might delay the onset of maximally effective treatment, effectively shortening the duration of exposure of *S. neurona* organisms in the CNS to effective concentrations of ponazuril.

One potential method to increase the CSF concentration of ponazuril is suggested by the observation that ponazuril is a highly lipophilic compound.⁴ If coadministration of ponazuril with oil enhances absorption of ponazuril this would be an economical means to enhance absorption and thereby increase serum and CSF concentrations, possibly improving effectiveness of treatment.

The purpose of the study was to determine if coadministration of corn oil and ponazuril would increase the concentration of ponazuril in serum and CSF concentration of adult horses.

2 | MATERIALS AND METHODS

2.1 | Animals

Ten mature adult horses of either sex, in good physical condition and with no evidence of systemic or neurological illness were used in our study. Horses were routinely vaccinated (Encevac TC-4, Bayer Animal Health, Merriam, Kansas) and dewormed (Eqvalan, Merck and Company, Rahway, New Jersey) before commencement of the study. To ensure that horses met inclusion criteria, all horses had a routine physical examination, were weighed, and a serum clinical chemistry analysis and complete blood cell count were performed. In addition, a neurological examination was performed by an experienced equine clinician (MF), and a CSF analysis including assessment of cell counts and total protein was performed. Horses were fed 0.45 kg of commercial sweet feed per day with locally sourced grass hay and water provided ad libitum. Animal welfare during the study was monitored by daily observation which was recorded on standardized forms.

Horses were randomly assigned to one of two study groups of 5 horses: those administered ponazuril (Marquis, Bayer Animal Health) (5 mg/kg BW PO), and with 50 mL of corn oil (PONOIL) once per day for 21 days, or those administered ponazuril (5 mg/kg BW PO) once per day for 21 days alone (PON). Horses were segregated by study group for housing in large paddocks for the duration of the study.

2.2 | Administration of ponazuril and oil

Horses assigned to the PONOIL group were administered 50 mL of corn oil PO using a 2 oz dose syringe placed into the horse's mouth through the interdental space and advanced over the caudal base of

the tongue. Corn oil was expelled and horses were allowed to swallow and then ponazuril was administered within 1-2 minutes in similar manner, using the dial-a-dose syringe provided and adjusted as appropriate for the horse's body weight. Horses were not fasted before administration, and concentrate was withheld for 2 hours after compounds were administered.

2.3 | Sample collection and processing

Blood was collected on study days 0, 7, 14, and 21 by jugular venipuncture using sterile evacuated tubes. Serum was harvested by aspiration after clot retraction and centrifugation at 1800g for 3 minutes. Routine clinical chemistry analysis was performed on study days 0 and 21 by the Clinical Laboratory of the Virginia Tech Equine Medical Center by an experienced laboratory technician, using automated equipment (Alfa-Wasserman VetAce Clinical Chemistry System, West Caldwell, New Jersey) that was calibrated daily. In addition, aliquots of serum were stored frozen at -65°C for future analysis.

Blood for CBC was collected on study days 0 and 21 into EDTA tubes and a routine analysis was performed by an experienced technician using an automated cell counter (Biochem Immunsystems Hematology, Montreal, Canada). A manual differential cell count (100 cells) was performed on Diff-Quick stained smears.

Cerebrospinal fluid was collected from the atlanto-occipital (AO) space using routine methods on study days 0, 7, 14, and 21. On study days 7, 14, and 21 collection occurred 1-2 hours after administration of ponazuril, whereas day 0 collection was 2 hours before administration of any test substance. In brief, horses were sedated with xylazine (0.5 mg/kg BW IV) (Sedazine, Bayer Animal Health) followed by ketamine (1.0 mg/kg BW IV) (Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa). After induction of anesthesia, the AO tap site was clipped and surgically prepared. Cerebrospinal fluid was collected using an 18 gage \times 3.5 in. styleted needle which was inserted and advanced until the dura was penetrated, at which time approximately 10 mL of CSF was collected by gentle aspiration. On day 0, a routine CSF analysis was performed within 1 hour of collection, which included red and white blood cell counts, as well as glucose and total protein concentration; cell counts were also conducted using freshly collected CSF on study days 7, 14, and 21. Remaining CSF was aliquoted by transfer to polypropylene tubes for storage at -65°C until analysis.

Aliquots of serum and CSF which had remained frozen were provided at the completion of the study for assay of ponazuril concentration using a previously validated analytical method.² Briefly, the sample was evaporated to dryness, dissolved in potassium carbonate solution, and passed through a disposable column with dichloromethane and ethyl acetate. Further cleanup was performed with a combination of an SCX-ion-exchange disposable column and a silica gel cartridge. The residue was dissolved in a high-performance liquid chromatography (HPLC) mobile phase and the concentration determined by HPLC analysis with fluorescence detection. Standards were evaluated using equine CSF or serum as the matrix as appropriate for the sample being assayed; the lower limit of detection was 0.014 mg/L for serum and

TABLE 1 Cerebrospinal fluid variables and serum and cerebrospinal fluid concentrations of ponazuril in 10 horses treated with ponazuril alone (PON), or ponazuril with corn oil (PONOIL)

	Day 7		Day 14		Day 21	
	PON, mean (SD)	PONOIL, mean (SD)	PON, mean (SD)	PONOIL, mean (SD)	PON, mean (SD)	PONOIL, mean (SD)
Red blood cell (cells/ μ L)	39.8 ^a (88.9)	2.2 ^a (2.68)	9.6 (9.58)	4.8 (7.52)	37.8 ^b (47.67)	1675.8 ^b (2056.8)
Albumin quotient	1.56 (0.32)	1.14 (0.054)	1.72 ^a (0.637)	1.06 ^a (0.114)	1.62 ^b (0.55)	1.22 ^b (0.164)
Ponazuril (mg/L) (CSF)	0.157 (0.347)	0.171 (0.008)	0.162 ^a (0.025)	0.213 ^a (0.036)	0.166 ^b (0.038)	0.243 ^b (0.043)
Ponazuril (mg/L) (serum)	4.35 ^a (0.759)	5.54 ^a (0.860)	4.42 ^b (0.443)	6.09 ^b (0.819)	4.65 ^c (0.901)	6.90 ^c (1.08)
Ponazuril S : CSF ratio	27.7 (1.6)	32.4 (4.2)	27.3 (2.2)	28.6 (2.4)	28.0 (5.1)	28.4 (2.3)

Note: Data are reported as mean and SD. Same superscripts within a row indicate significant difference ($P < .05$) for notated values.

0.008 mg/L for CSF. Further, CSF and serum albumin concentrations were determined by commercial laboratory (Equine Biodiagnostics, Inc, Lexington, Kentucky) and the albumin quotient (AQ) was calculated from the resulting data using standard formulas.⁵ All assayist were blinded to treatment group assignment.

2.4 | Data analysis

Data were entered into the statistical analysis program (SAS 9.4, SAS Institute, Cary, North Carolina) of a desktop computer for analysis. Data were summarized by calculating the mean and 1 SD of serum and CSF ponazuril concentrations by study day. Differences by treatment and study day were evaluated by repeated measures ANOVA; specific comparisons of interest were examined with contrast statements, with Tukeys' adjustment for posterior pair ways comparisons. As red blood cell (RBC) counts were not normally distributed, Friedmans test was used to determine if there were differences in RBC count by treatment group or study day. To determine if the CSF red blood cell count or AQ was influencing CSF ponazuril concentrations a correlation was performed. A P value of $<.05$ was set a priori for determination of statistical significance.

3 | RESULTS

Horses included in the study had no systemic or neurological abnormalities, and results of all clinical chemistry and CSF analyses were within normal limits at study commencement, and on day 21. Horses used in the study included 9 geldings and 1 non-gravid mare, ranging in age from 5 to 20 years (9.6 ± 6 ; median 9), and weighing from 461 to 652 kg (580.6 ± 58.5 , median 576). Breeds were 5 crossbreds, 1 Warmblood, 2 Quarter horses, and 2 Thoroughbreds.

Statistical analysis of the results of CBC and clinical chemistry analysis at day 0 found that there was no difference in any measured variable between the two treatment groups. Results for nucleated cell count, red blood cell count, and total protein of the CSF were within

normal limits for all horses, and group means did not differ between groups on day 0. The mean (± 1 SD) AQ in PON group horses was higher than that of horses in the PONOIL group (1.6 ± 0.23 versus 1.1 ± 0.19 ; $P = .02$) on day 0, however both values were within normal limits.⁵ Average plasma concentration of ponazuril at steady state (assumed to be days 7-21 based on the observation of concentration versus time results, as well as previous studies²) was 6.2 ± 0.9 mg/L (PONOIL) and 4.5 ± 1.0 mg/L (PON) ($P = .004$). Serum concentrations of ponazuril varied considerably, ranging from a 3.79-5.93 mg/L on study day 21 in horses given ponazuril only. Day 21 peak serum ponazuril concentrations in horses given oil and ponazuril ranged from 5.86 to 8.53 mg/L. Similarly, a large inter-horse variability was found in CSF ponazuril concentrations, ranging from 0.180 to 0.301 mg/L in those given ponazuril and corn oil, and from 0.134 to 0.169 mg/L in those given ponazuril alone. Average CSF concentrations at steady state (days 7-21) were 0.213 ± 0.04 mg/L (PONOIL) and 0.162 ± 0.04 mg/L (PON) ($P = .03$). Increasing CSF RBC concentrations were noted over time ($P = .03$), with differences between groups found on study days 7 and 21. The AQ did not differ by study day ($P = .96$) but differences were noted between treatment groups on some study days (0, 14, and 21). Results of correlation analysis of CSF ponazuril concentration and CSF RBC count ($R^2 = 0.254$; $P = .11$) and AQ ($R^2 = -0.042$; $P = .80$) found no association. Ponazuril concentrations in CSF and plasma over time are summarized in Table 1. The ratio of serum : CSF concentrations varied from 27.7 to 32.4 for study days 7-21; the ratios did not differ between treatment groups ($P = .16$) or study day ($P = .33$).

4 | DISCUSSION

Results of the study found that coadministration of corn oil and ponazuril resulted in higher serum concentration on treatment days 7, 14 and 21. This was associated with a higher CSF ponazuril concentration on days 14 and 21, but not on day 7. These results suggest that the higher CSF concentrations are solely because of the higher serum concentrations of ponazuril. Consistent with previous studies,²

the serum : CSF ponazuril concentration ratio did not change on study days 7, 14, and 21 period, indicating that there was no accumulation of ponazuril in the CSF.

The CSF ponazuril concentration was not significantly higher after 7 days of dosing, suggesting that equilibration with the CSF might take longer than 7 days. If this is correct, then strategies based on increasing serum concentration might have limited effectiveness in achieving higher CSF concentrations more quickly. However, review of the data finds that the SD for the ponazuril-only group on day 1 had a large SD, because of the results of one individual horse, which might have biased the results. This indicates a weakness of the study which is the fairly small sample size.

There appears to be substantial inter-horse variability in pharmacokinetics of ponazuril. At 7 days of treatment, some horses in our study had CSF concentrations which were only slightly greater than the effective ponazuril concentration,³ particularly associated with large variability in the ponazuril only group and substantially lower serum concentrations after 7 days of treatment. Such horses in particular are likely to benefit from strategies to increase CSF concentration.

The AQ is a ratio of the concentrations of albumin in the CSF and serum, and is used as a measure of blood brain barrier (BBB) permeability.⁵ It is possible that repeated spinal fluid collection procedures might increase BBB permeability and thus influence the movement of ponazuril into the CSF. Values in our study for AQ were within reported normal values (1.4 ± 0.4)⁵ for all horses throughout the study. While the AQ did not change over time with repeated collections, it did differ by study group, and was consistently higher in the ponazuril-only group. Reasons for this are not obvious from the results of the study, but cannot be because of the effects of test article administration, as the difference was observed on study day 0, before drug administration had begun. It is more likely that this is simple individual animal variation, and the effect remained consistent throughout. Given that the values for all animals were within normal limits, it is unlikely that this had an effect upon the study results. If the increased AQ represented significantly greater BBB permeability and hence greater movement of ponazuril across the BBB, the results would have been biased towards increasing CSF ponazuril concentrations and reducing the likelihood of finding a treatment effect. This is opposite to the observed findings.

An additional factor that might have altered the CSF results was the CSF RBC count. Increasing counts would suggest blood contamination of the CSF because of repeated punctures, or perhaps direct contamination of the CSF with blood containing high concentrations of ponazuril. Increasing RBC counts were observed over time during the study, with the ponazuril and oil study group having more RBCs on day 21. This was due in large part to one individual horse with a

particularly high RBC count. Analysis did not find correlation of RBC count and CSF ponazuril concentration, suggesting that these factors had little effect upon CSF ponazuril concentrations.

Results of the current study indicate that coadministration of corn oil and ponazuril leads to higher serum and CSF ponazuril concentrations. It is unclear if this effect is adequate to result in improved treatment efficacy, yet this approach appears to be an effective and inexpensive means to increase ponazuril concentrations in the serum and CSF.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Study protocol approved by the Virginia Tech IACUC.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for our study.

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