CONFIDENTIAL PRELIMINARY DRAFT FOR REVIEW

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CLINICALY EFFECTIVE LOADING DOSE SCHEDULE FOR PONAZURIL® IN THE HORSE.

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Summary:

Pharmacokinetic modeling suggests that administration of two 20 mg/kg loading doses of Marquis® [ponazuril] on days 1 and 2 of treatment will dramatically accelerate attainment of therapeutically effective plasma concentrations of Marquis®. These findings are consistent with the senior author's experience that this loading dose approach significantly accelerates the onset of the therapeutic effects of Marquis® in the treatment of Equine Protozoal Myeloencephalitis.

Background:

Marquis® [ponazuril] is an oral paste formulation of ponazuril that is highly effective in the treatment of Equine Protozoal Myeloencephalitis [EPM] [1, 2, and 3]. The manufacturer's recommended dose is 5 mg/kg daily, for 28 days [1]. Ponazuril is well absorbed following oral administration and has a relatively long [2-3 day] plasma half-life (4, 5). As such, once attained, therapeutically effective plasma concentrations

of ponazuril are readily maintained with minimal daily peak to trough plasma concentration variation (5, 6).

On the other hand, this long plasma half-life of ponazuril means that it can take up to seven days or longer to attain therapeutically effective blood levels following the manufacturers recommended dose of 5 mg/kg/day. The pharmacokinetic rule of thumb for time to attain steady-state concentrations of any medication is four to five plasma half-lives, in the order of 8 or more days for ponazuril. Given this circumstance, one of us [S.M. Reed] routinely administers two 20 mg/kg loading doses of Marquis® on days 1 and 2 of the 28-day treatment schedule, with the goal of accelerating the attainment of therapeutically effective plasma concentrations of ponazuril.

While apparently effective, this loading dose strategy has never been scientifically validated. We now report an "in silico" pharmacokinetic analysis showing that therapeutically effective plasma concentrations of Ponazuril are rapidly attained following the Reed loading dose schedule, with resultant therapeutic benefits for the horse, and most especially in the matter of acute episodes of EPM.

Pharmacokinetic analysis:

These pharmacokinetic projections are based on the pharmacokinetic data reported for ponazuril in the horse by Dirikolu et al [4, 5]. In this model the steady-state ($C_{ss\ ave}$) of ponazuril was determined based on plasma clearance of ponazuril, since clearance (Cl) is the major determinant of the eventual $C_{ss\ ave}$ concentrations achieved following a maintenance dose of 5 mg/kg/day for 28 days. Using Dirikolu's oral administration clearance value, Cl (mL/h) = 3111.4 mL/h (74.66 L/day) and assuming a maintenance dose of 5 mg/kg/day with a dosing interval (τ) of day and an oral bioavailability (F) of ponazuril of 0.71, the oral clearance (Cl) is 74.664 L/day and the final steady state concentration of ponazuril was calculated as 23.94 =24 ug/ml, as set forth in figure #1below.

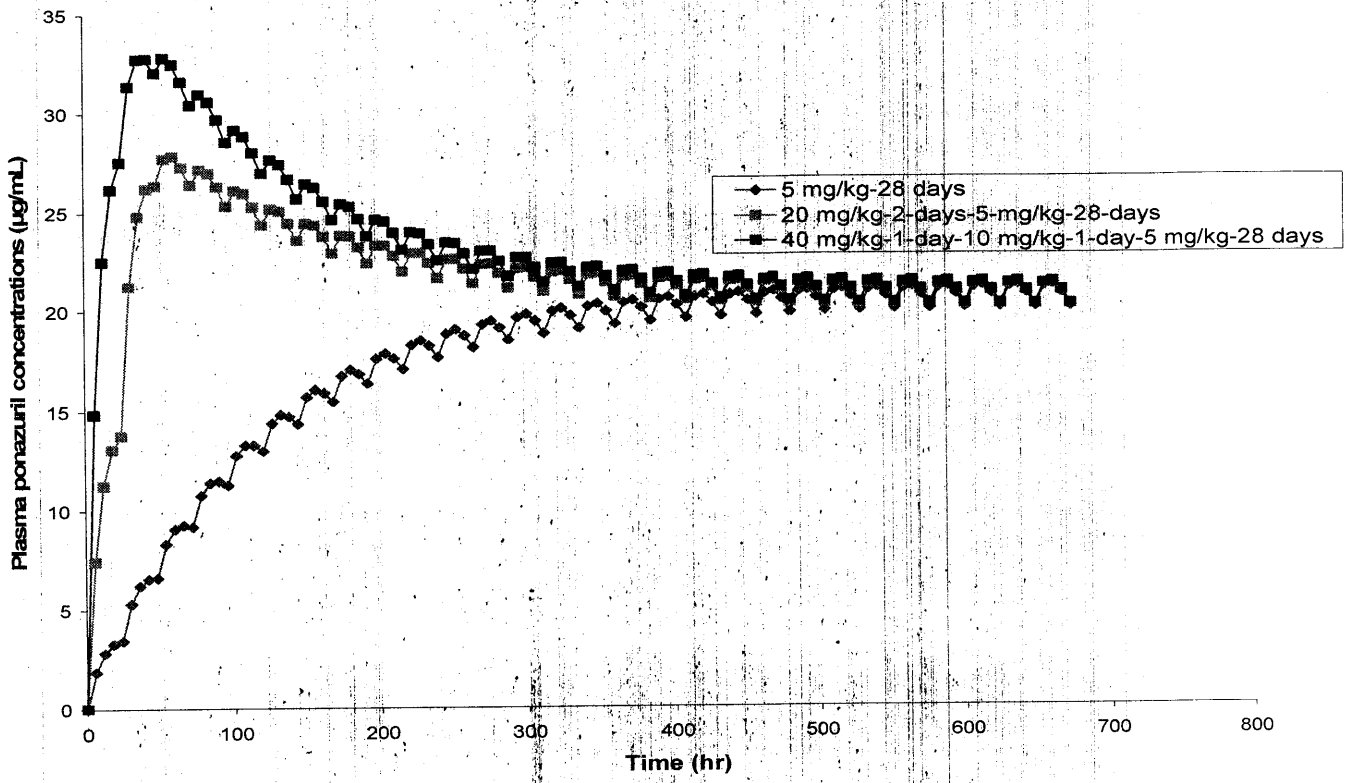

Figure 1: Predicted plasma concentrations of ponazuril (µg/mL) after treatment of horses with ponazuril following: maintenance dose of 5 mg/kg/day for 28 days (red diamonds); loading dose of 20 mg/kg/day for 2 days followed by a maintenance dose of 5 mg/kg/day for 28 days (green squares); loading dose of 40 mg/kg on day 1, 10 mg/kg on day 2, and maintenance dose of 5 mg/kg/day for 28 days (blue square).

Results and discussion:

As shown by the red symbols in figure 1, when dosing starts at 5 mg/kg/day it takes at least seven days to approach therapeutically effective steady-state plasma concentrations of ponazuril, which are in the order of 20-25 ug/ml. This slow attainment of the steady-state concentration of ponazuril creates a classic loading dose situation [7]; the green symbols show the calculated plasma levels of ponazuril following the Reed loading dose schedule of 4X doses on days one and two. Following administration of the first 20 mg/kg [4X] loading dose on day one, plasma concentrations of Ponazuril peaked at about 15 mg/ml at 24 hours, at which point the second 20 mg/kg loading dose is administered. At 48 hours, 24 hours following this second loading dose administration, the plasma concentrations of Ponazuril reach 27 mg/ml, fractionally above the target therapeutic plasma level of ponazuril Thereafter,

dosing returns to the manufacturer' s recommended 5 mg/kg/day and the final therapeutic steady-state plasma concentration of ponazuril is approached, but this time from a higher value, rather than more slowly from lower plasma concentrations as when the manufacturer's dosing schedule is followed.

We also elected to estimate the plasma concentrations of ponazuril after a day one 8X loading dose [40 mg/kg], followed by a day 2 dose of 10 mg/kg. As shown by the blue symbols in figure 1, this loading dose schedule considerably overshot [33 mcg/ml at day 2] the eventual steady-state plasma concentration of ponazuril.

Although a 4X loading dose of 20 mg/kg may seem large, it is clear that even following this first 4X dose the full steady-state plasma concentration of ponazuril is not attained. As such, administration of this loading dose is essentially risk-free, and an additional factor which make this loading dose approach both safe and effective is the essential non-toxicity of ponazuril in the therapeutic concentration range.

In a clinical situation, and most especially in an acute clinical situation, the rapid attainment of therapeutically effective concentrations of ponazuril must be seen as a considerable therapeutic advantage. On the other hand, there are no apparent disadvantages to this approach. The plasma concentrations obtained following the second loading dose are fully therapeutic and only fractionally above the eventual steady-state therapeutic concentrations. As such there is essentially zero risk of a dose-related adverse response, but consider potential for therapeutic benefit from the rapid attainment of optimally therapeutically effective blood concentrations of ponazuril, consistent with Dr. Reed's clinical experience with this simple, safe, and highly effective loading dose schedule.

Literature cited:

1) www.bayerdvm.com/Products/marquis/marquis.cfm - 21k

2) Bentz, B.G., Dirikolu, L., Carter, W.G., Saville, W., Bernard, W.V., Wulff-Strobel, C., McCrillis, S., Reed, S., Granstrom, D.E., Tobin, T., (2000) Clinical report on diclazuril and equine protozoal myeloencephalitis (EPM). *Equine Vet. Education,* 2:258-263, [KY Ag Exp Sta #98-14-159].

3) Bentz, B.G., Carter, W.G., Tobin, T., (1999) Equine protozoal myeloencephaltis (EPM): Review of a diagnostic approach. *Compendium on Continuing Education for Practicing Veterinarians*, 21:975-981, [KY Ag Exp Sta #98-14-158].

4) Dirikolu, L., Lehner, A.F., Hughes, C., Karpiesiuk, W., and Tobin, T., (2008) New Therapeutic Approaches to Equine Protozoal Myelitis: Pharmacokinetics of Toltrazuril Sulfone Sodium Salts in the Horse AAEP Proc. Vol 54 pp 191

5) Dirikolu, L., Karpiesiuk, W., Lehner, A.F., Hughes, C. Granstrom, D.E. Tobin, T. (2009) Synthesis and Detection of Toltrazuril Sulfone and Its Pharmacokinetics in Horses Following Administration in Dimethylsulfoxide. *Journal of Veterinary Pharmacology and Therapeutics*; 32, (4) pp. 368-378.

6) Furr, M., McKenzie, H., Saville, W.J.A., Dubey, J.P., Reed, SM., and Davis, W., (2006) Prophylactic Administration of Ponazuril Reduces Clinical Signs and Delays Sero-conversion in Horses Challenged with Sarcocystis Neurona. *J. Parasitol.* 92 (3) pp 637-643.

7) *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 10th ed.; Hardman, J. G., Limbird, L. E., Eds.; McGraw-Hill: New York, 2001.