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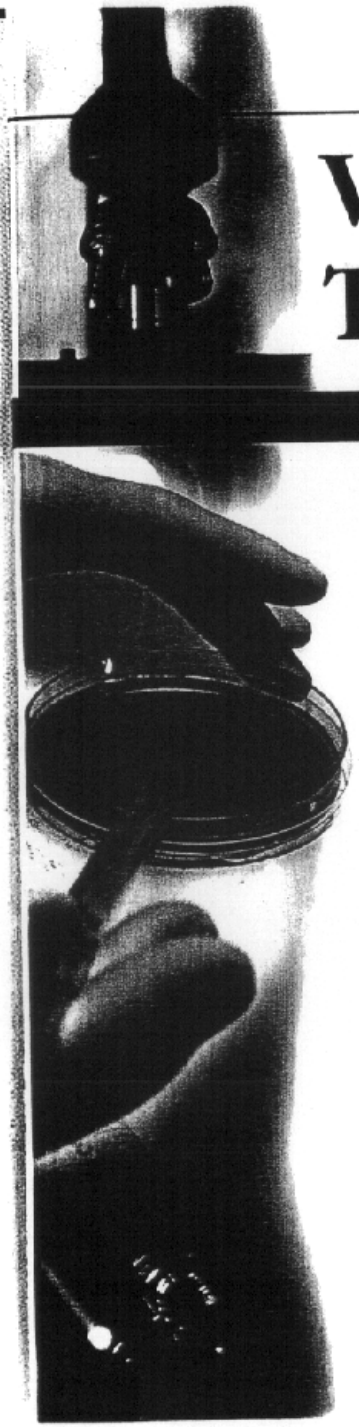

New Therapeutic Approaches for Equine Protozoal Myeloencephalitis: Pharmacokinetics of Diclazuril Sodium Salts in Horses*

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CLINICAL RELEVANCE

Diclazuril, a triazine-based antiprotozoal agent, may have clinical application in the treatment of equine protozoal myeloencephalitis (EPM). Diclazuril was rapidly absorbed, with peak plasma concentrations occurring at 8 to 24 hours after oral-mucosal administration of diclazuril sodium salt. The mean oral bioavailability of diclazuril as Clinacox was 9.5% relative to oral-mucosal administration of diclazuril sodium salt; diclazuril in dimethyl sulfoxide administered orally was 50% less bioavailable than with oral-mucosal administration of diclazuril sodium salt. Diclazuril sodium salt has the potential to be used as a feed additive for the treatment and prophylaxis of EPM and various other apicomplexan-mediated diseases.

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INTRODUCTION

The neurologic disease now known as *equine protozoal myeloencephalitis* (EPM) was first discussed in Philadelphia.[1-2] Forty-four cases of what came to be known as EPM were identified in the 1960s.[2] The causative parasite, *Sarcocystis neurona* (Phylum: Apicomplexa), was isolated for the first time from the spinal cord of a horse in bovine monocyte cell cultures.[3] More recently, a similar neurologic disease has been described in horses from which *Neospora* spp have been isolated.[4] Even though the organism that causes this disease is recognized by antibodies against *Neospora caninum*, it has been reported that it is a new species, *Neospora hughesi*, and differences in the amino acid sequence of two immunodominant surface antigens support suggestions that this organism is a new etiologic agent in equines.[5]

The epidemiologic and economic significance of *S. neurona* infection is substantial. In endemic areas of the United States, 45% to 60% of horses are seropositive for this protozoan.[6,7] Of animals clinically affected, 30% to 40% reportedly fail to respond to current therapy (pyrimethamine–sulfonamide combinations), and some of these animals die.[6] While this combination therapy is successful in many cases, the treatment can be prolonged and the occurrence of relapses after cessation of treatment is common.[6] Current treatments also carry significant toxicity risks,[8-9] and therefore, safer, more effective, and less toxic prophylactic and therapeutic procedures are desirable.

Diclazuril (2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3*H*) yl)benzeneacetonitrile) is a triazine-based antiprotozoal agent. Previous studies have identified triazine-based antiprotozoal agents for the treatment and prophylaxis of EPM in the horse.[6,10,11] On this basis, we elected to develop a highly bioavailable oral formulation of diclazuril, namely diclazuril sodium salt. The study described here evaluated the bioavailability of an orally administered sodium salt formulation of diclazuril for the treatment and prophylaxis of EPM.

MATERIALS AND METHODS

Synthesis of Diclazuril Sodium Salt

Diclazuril powder (100 g) was obtained from New Ace Research (Versailles, KY). A freshly prepared solution of sodium ethanolate (NaOEt; obtained from 1.18 g, 1.05 mol eq of sodium) in 100 ml absolute ethanol (EtOH) was slowly added to a hot suspension of 20 g of diclazuril in 300 ml absolute ethanol, keeping the color of the reaction mixture light brown. After stirring for 1.5 hours at 70°C, the solvent was evaporated under reduced pressure and the residue was dried under high (>1 mm Hg) vacuum to obtain 21.2 g (100% purity determined by thin layer chromatography) of diclazuril sodium salt as an amorphous brownish powder. The obtained salt is very soluble in water, giving an almost neutral solution (Figure 1).

Horses and Sample Collection

Horses were provided by Saxony Farm (Versailles, KY) and were maintained on grass hay and a 50:50 mixture of oats and an alfalfa-based protein pellet; horses were fed twice daily. The animals were vaccinated annually for tetanus and dewormed quarterly with ivermectin (obtained from MSD Agvet, Rahway, NJ). Horses were kept in a 20-acre field until they were placed in box stalls, where they were provided water and hay ad libitum. Horses were not fed for at least 2 hours before and 1 hour after oral administration of each drug formulation included in this study. The horses were managed according to the rules and regulations of the University of Kentucky Institutional Animal Care and Use Committee, which approved the experimental protocol.

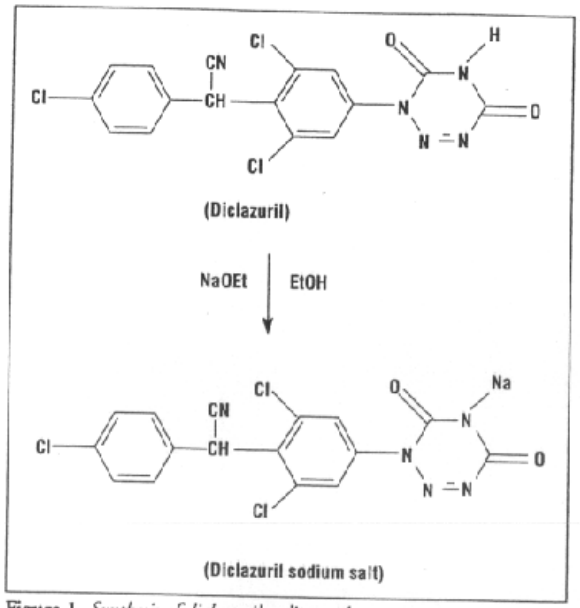

Figure 1. *Synthesis of diclazuril sodium salt.*

In this study, four groups of four horses each received one of the following oral formulations of diclazuril:

- 5 mg/kg diclazuril as Clinacox (Pharmacia-Upjohn, Ontario, Canada)
- 2.2 mg/kg of diclazuril sodium salt
- 2.2 mg/kg of diclazuril in dimethyl sulfoxide (DMSO)
- 2.2 mg/kg diclazuril sodium salt as a feed additive in 0.5 oz beet pulp added to 1 lb sweet feed

Four mature Thoroughbred mares weighing 518 to 564 kg received diclazuril sodium salt by direct application of 2.2 mg/kg on the oral mucosa. Blood samples for analysis were collected in heparinized tubes from the right jugular vein at 0, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours. All samples were centrifuged at 4°C and 2,000 ×*g* for 15 minutes, and the plasma was aspirated and stored at –20°C until assayed.

Four mature Thoroughbred mares weighing 495 to 536 kg were used for determination of the usefulness of diclazuril sodium salt as a feed additive and received 2.2 mg/kg diclazuril sodium salt in 0.5 oz beet pulp added to 1 lb sweet feed. Blood samples for analysis were obtained from the right jugular vein at 0, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours; plasma samples were prepared and stored as described above.

Four mature Thoroughbred mares weighing 461 to 576 kg received oral diclazuril as Clinacox, a poultry feed premix containing 0.5% diclazuril and 99.5% protein carrier.[11] It was administered by nasogastric intubation at a single dose of 5 mg/kg diclazuril suspended in 6 to 8 L of water. Plasma samples were collected, prepared, and stored as described above.

Four mature Thoroughbred mares weighing 480 to 556 kg received 2.2 mg/kg diclazuril in DMSO. Diclazuril solution was prepared at 100 mg/ml concentration in DMSO, and approximately 11 to 13 ml of this solution was administered orally using 15-ml syringes. Plasma samples were collected, prepared, and stored as described above.

Diclazuril Analysis

Sample Preparation

Diclazuril was analyzed using high-pressure liquid chromatography (HPLC) as described elsewhere.[11] A standard solution of 1 mg diclazuril (Janssen compound R 64433) was prepared in 1 ml HPLC-grade dimethylformamide

(DMF) (Sigma-Aldrich 27,054-7). Working standards at 0, 0.25, 0.5, 0.75, 1, 2.5, 5, and 10 µg/ml were prepared by adding specific amounts of the stock diclazuril standard at 0.01, 0.1, and 1 µg/µl in DMF:water (1:1) to 1-ml aliquots of diclazuril-free horse plasma. Janssen compound R 62646, a structural analog of diclazuril, was used as the internal standard, which was prepared in 1 ml DMF (1 mg/ml) and diluted 1 to 10 in DMF:water (1:1) to yield a 0.1 µg/µl standard solution; 20 µl of the internal standard solution and 2 ml of 0.1 M potassium phosphate buffer (pH 6.0) were added to 1 ml of plasma sample or standard.

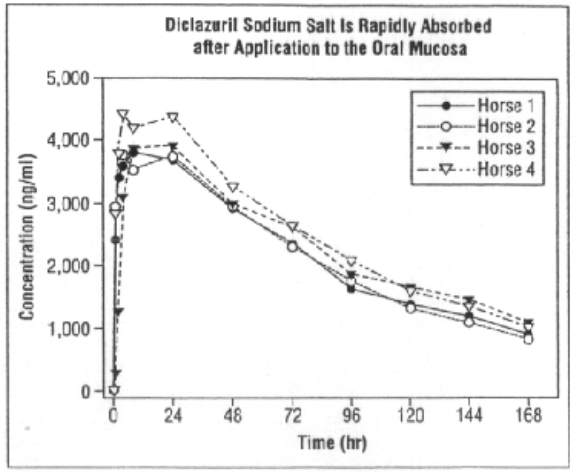

Figure 2. *Plasma concentrations of diclazuril from four horses following a single oral-mucosal administration of diclazuril sodium salt (2.2 mg/kg).*

Extraction Method

Mega Bond Elut C18 columns (Varian, Harbor City, CA) were treated sequentially with 2 ml of HPLC-grade methanol and 2 ml of 0.1 M potassium phosphate buffer (pH 6.0) under light vacuum. Prepared plasma samples were drawn slowly through the column, and the column was sequentially rinsed with 2 ml of 0.1 M potassium phosphate buffer (pH 6.0), 2 ml of 1.0 M acetic acid, and 2 ml of hexane. The column was allowed to dry for 10 minutes after each rinse. The column was eluted with 4 ml of methanol:HCl (95:1), and the eluent was collected in a tapered silanized glass tube; the solvent was then evaporated under a stream of nitrogen at 40°C. The residue was resuspended first in 100 µl of DMF with moderately vigorous vortexing and sonication, mixed with 100 µl of water, and placed into a 300 µl vial for HPLC analysis.

The limit of detection (LOD) was defined using the analyte's peak height compared with the baseline noise in the chromatogram. By this method, the LOD was defined as the lowest concentration of analyte producing a peak greater than or equal to three times the baseline noise of the ion chromatogram. The lower limit of quantitation (LOQ) was defined as the concentration calculated from the mean of the zero responses plus five times the standard deviation.[12] The extraction efficiency was determined by comparing the response (in area) of 1,000 ng/ml and internal standard (2 µg/ml) standards spiked to blank plasma eluent before evaporation to the equivalent extracted standards over six runs.

Instrumentation

The HPLC procedure was adapted from that described elsewhere.[13] The instrument used was a Beckman System Gold HPLC (Beckman, Palo Alto, CA) with two 110B solvent delivery pumps, a 168 photodiode array detector, and a 502 autosampler. The column was a Beckman Ultrasphere ODS, 5 µm particle size, 4.6 mm × 15 cm

TABLE 1. Pharmacokinetic Parameters of Diclazuril after a Single Oral-Mucosal Administration of Diclazuril as a Sodium Salt (2.2 mg/kg)[a]

	Horse				
Parameter	*1*	*2*	*3*	*4*	*Mean (±SD)*
Weight (kg)	518	555	564	527	541 (±22)
$t_{1/2}$ K_{01} (hr)	0.81	0.579	3.263	0.802	1.36 (±1.27)
$t_{1/2}$ K_{10} (hr)	80	75	75	80	77.5 (±2.88)
$AUC_{0\text{-}inf}$ (ng/ml/hr)	477,694.88	456,104	513,723	552,932	500,113 (±42,483)
Oral clearance (L/hr)	2.38	2.65	2.41	2.09	2.38 (±0.23)
T_{max} (hr)	5.52	4.09	15	5.4	7.5 (±5)
C_{max} (ng/ml)	3,944	4,052	4,123	4,559	4,170 (±270)
r^2	0.99	0.98	0.98	0.99	0.99

[a]Using a nonlinear regression program (Winnonlin, version 4.01).

TABLE 2. Pharmacokinetic Parameters of Diclazuril after a Single Oral Administration as Clinacox (5 mg/kg)[a]

	Horse				
Parameter	*1*	*2*	*3*	*4*	*Mean (±SD)*
Relative F (%)[b]	5	11	14	7	9.25 (±4)
$t_{1/2}$ K_{01} (hr)	11.79	6.45	8.16	6.38	8.2 (±2.53)
$t_{1/2}$ K_{10} (hr)	25.69	59.93	43.55	40.38	42.4 (±14)
$AUC_{0\text{-}inf}$ (ng/ml/hr)	56,069.54	115,158.6	145,980.94	78,301	98,877 (±39,748)
Oral clearance (L/hr)	44.58	21.71	17.13	31.93	28.9 (±12.2)
T_{max} (hr)	24.5	23.3	24.3	20.2	23.05 (±1.99)
C_{max} (ng/ml)	756.42	1,007.5	1,570	974	1,077 (±347)
r^2	0.99	0.994	0.996	0.99	0.99

[a]Using a nonlinear regression program (Winnonlin, version 4.01).
[b]Relative bioavailability (F) was calculated using diclazuril sodium salt as a reference.

column. The mobile phase consisted of 46% solvent A and 54% solvent B run with a flow rate of 1 ml/min isocratically. Solvent A was 80% (0.5% ammonium acetate, 0.01 M tetrabutylammonium hydrogen sulfate [Sigma # 39684-2] in water):20% acetonitrile. Solvent B was 80% methanol:20% acetonitrile. The diode array detector was set up for single wavelength acquisition at 280 nm with a 12-nm bandwidth. Injections were made with a 20-µl loop.

Pharmacokinetic Analysis

Pharmacokinetic analyses were performed using a nonlinear regression program (Winnonlin, version 4.01; Pharsight Corporation, Cary, NC). The area under the plasma drug concentration versus time curve (AUC) was measured by use of a linear trapezoidal approximation with extrapolation to infinity. The slope of the terminal portion (K_{10}) of this curve was determined by the method of least-squares regression.[14]

The single compartmental model used is represented by general equation *a* (see box on page 58 for the equations used in the pharmacokinetic analyses). The rate constant of absorption (K_{01}) and the absorptive half-life ($t_{1/2}$ K_{01}) were determined using the method of residuals. The terminal elimination half-life ($t_{1/2}$ K_{10}) was calculated according to equation 1. Total oral clearance (Cl_s) was calculated by equation 2. The maximum drug concentration af-

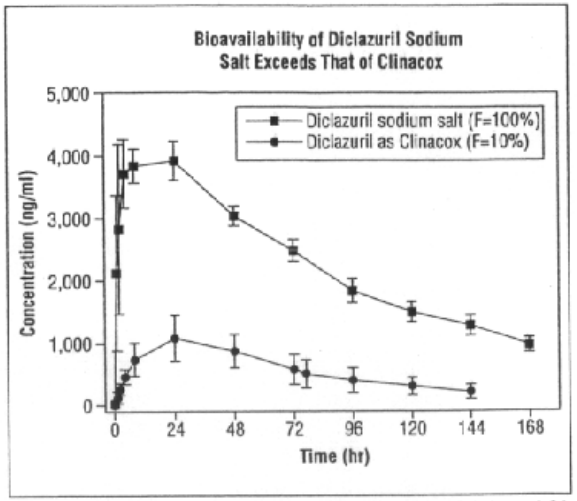

Figure 3. *Comparison of mean plasma concentrations of diclazuril following a single oral administration as Clinacox (5 mg/kg; n = 4) and a single oral-mucosal administration of diclazuril sodium salt (2.2 mg/kg; n = 4). Relative bioavailability (F) was calculated using diclazuril sodium salt as a reference.*

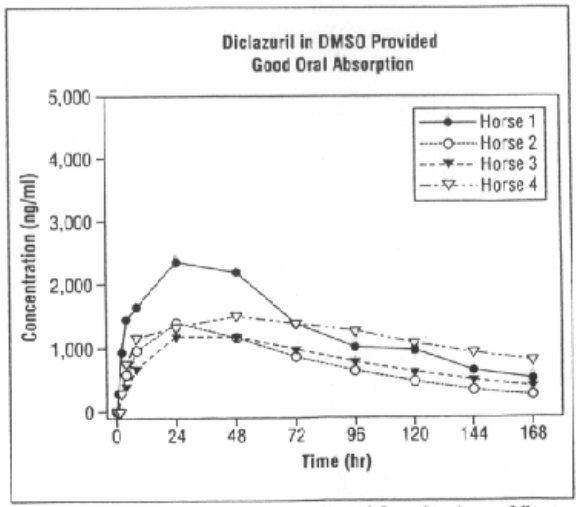

Figure 4. *Plasma concentrations of diclazuril from four horses following a single oral administration of diclazuril in DMSO (2.2 mg/kg).*

Equations Used in Pharmacokinetic Analyses

General Equation *a*—Single compartmental model used

$$Cp = (A \times e^{-K_{01}t}) - (A \times e^{-K_{10}t})$$

Cp is the plasma concentration of compound at any time point (t), A is the Y intercept associated with the terminal elimination phase, K_{01} is the apparent rate constant of absorption, and K_{10} is the apparent rate constant of elimination.

The rate constant of absorption (K_{01}) and the absorptive half-life ($t_{1/2}\,K_{01}$) were determined using the method of residuals.

Equation 1—Terminal elimination half-life ($t_{1/2}\,K_{10}$)

$$t_{1/2}\,K_{10} = \frac{\ln 2}{K_{10}}$$

Equation 2—Total oral clearance (Cl_o)

$$Cl_o = \frac{\text{Dose (oral)}}{AUC_{0-\infty}}$$

Equation 3—Maximum drug concentration after oral administration (C_{max})

$$C_{max} = (A \times e^{-K_{10}T_{max}}) - (A \times e^{-K_{01}T_{max}})$$

Equation 4—Time at which C_{max} was achieved (T_{max})

$$T_{max} = \left(\frac{1}{K_{01} - K_{10}}\right) \times \left(\ln \times \frac{K_{01}}{K_{10}}\right)$$

Equation 5—Relative bioavailabilities (F) of diclazuril

$$F = \frac{AUC_{0\text{-}inf}\ \text{(diclazuril sodium salt)}}{AUC_{0\text{-}inf}\ \text{(other formulations)}} \times \frac{\text{Dose (other formulations)}}{\text{Dose (diclazuril sodium salt)}}$$

ter oral administration (C_{max}) and the time at which C_{max} was achieved (T_{max}) were determined by equations 3 and 4, respectively. The relative bioavailabilities (F) of diclazuril as Clinacox, in DMSO, and as a feed additive were calculated from the $AUC_{0\text{-}inf}$ ratio comparison with the diclazuril sodium salt by equation 5.

■ RESULTS

The HPLC diode array detection method reported here readily detects diclazuril in plasma, with an LOD of diclazuril in plasma of about 5 ng/ml and LOQ of diclazuril in plasma of about 10 ng/ml. Satisfactory recovery (82% ± 5% SD) was obtained for solid-phase extraction of diclazuril from plasma samples of horses by HPLC. The diclazuril peak eluted at around 13.00 (±0.8) minutes, and the internal standard peak eluted at 14.50 (±0.8) minutes.[11] The peaks were symmetric, and the standard curve was linear from 0.25 to 10 µg/ml with an r^2 of 0.9998 (data not shown). The areas of the peaks corresponding to diclazuril and internal standard were recorded, and the internal standard values were used to normalize the diclazuril areas. Integrated peak values were entered into QuattroPro for Windows for statistical analysis of standards and for interpolation of unknown amounts of diclazuril. Standard curves were generated with Sigma Plot for Windows.

Analysis of the plasma samples showed rapid absorption of diclazuril following application of diclazuril sodium salt to the oral mucosa (Figure 2). Peak plasma concentrations of 3,930 (±308 SD) ng/ml of diclazuril were observed 8 to 24 hours after administration, and the observed peak plasma concentrations were in close agreement. Thereafter, observed plasma concentration declined to 964 (±116 SD) ng/ml at 168 hours after administration, with an apparent average elimination half-life of approximately 78 hours. Pharmacokinetic parameters of diclazuril sodium salt following oral-mucosal administration are shown in Table 1.

TABLE 3. Pharmacokinetic Parameters of Diclazuril in DMSO after a Single Oral Administration (2.2 mg/kg)[a]

	Horse				
Parameter	*1*	*2*	*3*	*4*	*Mean (±SD)*
Relative F (%)[b]	52	30	38	76	49 (±20)
Weight (kg)	563	536	482	546	531.5 (±35)
$t_{1/2}\,K_{01}$ (hr)	5.05	7.24	10.32	5.47	7.02 (±2.4)
$t_{1/2}\,K_{10}$ (hr)	63	54	71	158	86.5 (±48)
$AUC_{0\text{-}inf}$ (ng/ml/hr)	270,537.52	147,112	169,290.71	379,801	241,690 (±106,604)
Oral clearance (L/hr)	4.07	8.02	6.26	3.16	5.38 (±2.19)
T_{max} (hr)	20	24.2	34	27.5	26.43 (±5.9)
C_{max} (ng/ml)	2,429	1,398	1,238	1,526	1,647.8 (±534)
r^2	0.99	0.98	0.99	0.99	0.99

[a]Using a nonlinear regression program (Winnonlin, version 4.01).
[b]Relative bioavailability (F) was calculated using diclazuril sodium salt as a reference.

After administration of a single oral dose of diclazuril (as Clinacox) to four horses, analysis of plasma samples showed detectable plasma concentrations of diclazuril,[11] with a mean peak plasma concentration of 1,077 (±348 SD) ng/ml of diclazuril at 24 hours after administration (data not shown). Thereafter, the plasma concentration declined to 208

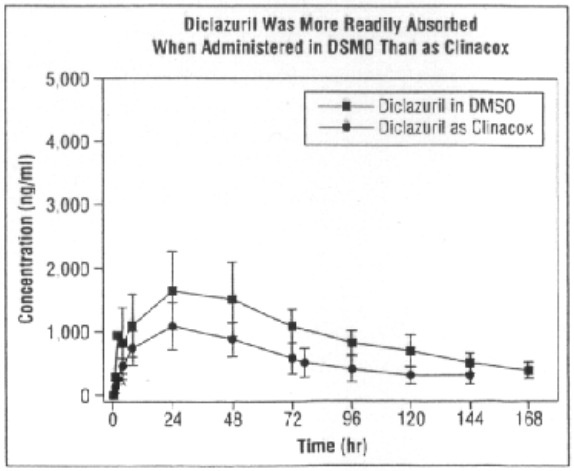

Figure 5. *Comparison of mean plasma concentrations of diclazuril following single oral administrations as Clinacox (5 mg/kg; n = 4) and diclazuril in DMSO (2.2 mg/kg; n = 4).*

TABLE 4. Pharmacokinetic Parameters of Diclazuril after a Single Oral Administration of Diclazuril Sodium Salt as a Feed Additive[a]

	Horse				
Parameter	*1*	*2*	*3*	*4*	*Mean (±SD)*
Relative F (%)[b]	52	34	41	54	45.25 (±9.42)
Weight (kg)	527	495	536	518	519 (±17.6)
$t_{1/2}K_{el}$ (hr)	0.7	3.64	0.848	3.06	2.06 (±1.51)
$t_{1/2}K_{10}$ (hr)	50	47	64.5	52	53.38 (±7.7)
$AUC_{0\text{-inf}}$ (ng/ml/hr)	252,565	156,594	201,650	258,451	217,320 (±47,844)
Oral clearance (L/hr)	4.59	6.95	5.85	4.41	5.45 (±1.18)
T_{max} (hr)	4.36	14.54	5.37	13.31	9.4 (±5.27)
C_{max} (ng/ml)	3,270	1,867	2,047	2,870	2,514 (±667)
r^2	0.99	0.92	0.98	0.92	0.95

[a]Using a nonlinear regression program (Winnonlin, version 4.01).
[b]Relative bioavailability (F) was calculated using diclazuril sodium salt as a reference.

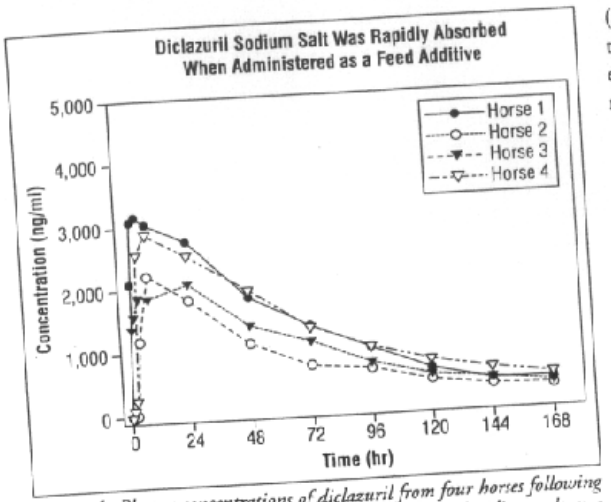

Figure 6. *Plasma concentrations of diclazuril from four horses following a single oral administration of 2.2 mg/kg of diclazuril sodium salt as a feed additive (in 0.5 oz beet pulp added to 1 lb sweet feed).*

(±116 SD) ng/ml at 144 hours after administration, with an apparent average half-life of approximately 43 hours.[11]

The pharmacokinetic parameters of diclazuril following administration as Clinacox are shown in Table 2. Figure 3 shows a comparison of the mean plasma concentrations of diclazuril after oral administration as Clinacox (5 mg/kg)[11] and after oral-mucosal administration of diclazuril sodium salt (2.2 mg/kg). The relative oral bioavailabilities of diclazuril as Clinacox ranged from a low of 5% to a high of 14% compared with oral-mucosal administration of diclazuril sodium

salt, with a mean relative oral bioavailability of about 10%. The peak plasma concentration of diclazuril as Clinacox was four times less than that of diclazuril as a sodium salt.

After administration of a single oral dose of diclazuril (2.2 mg/kg) in DMSO to four horses, analysis of plasma samples showed good oral absorption of this compound (Figure 4), with an observed mean peak plasma concentration of 1,645 (±616 SD) ng/ml of diclazuril at 24 to 48 hours after oral administration. Thereafter, the plasma concentration declined to 383 (±128 SD) ng/ml at 168 hours after administration, with an apparent average half-life of 87 hours. One horse (number 4) had a relatively long plasma half-life of diclazuril (158 hours) (Table 3). The samples from this horse were analyzed three times to confirm the results; all three analyses provided similar pharmacokinetic parameters, indicating variability of diclazuril metabolism among horses. Observed peak plasma concentrations from these horses were relatively closely distributed, ranging from a low of 1,190 ng/ml to a high of 2,347 ng/ml (Figure 4).

Figure 5 shows the comparison of the mean plasma concentrations of diclazuril following oral administration of 2.2 mg/kg in DMSO and 5 mg/kg as Clinacox. The observed mean peak plasma concentration of diclazuril at 24 hours after oral administration in DMSO was approximately 1.5 times higher than that after oral administration as Clinacox. The relative oral bioavailability of diclazuril as Clinacox compared with that of diclazuril in DMSO was 20%, indicating an approximate fivefold reduction in bioavailability of diclazuril following oral administration as Clinacox versus in DMSO.

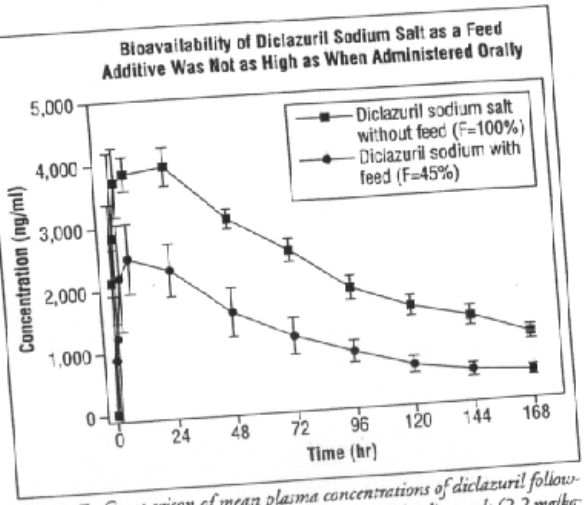

Figure 7. *Comparison of mean plasma concentrations of diclazuril following single oral-mucosal administration of diclazuril sodium salt (2.2 mg/kg; n = 4) and oral administration of diclazuril sodium salt as a feed additive (in 0.5 oz beet pulp added to 1 lb sweet feed; n = 4). Relative bioavailability (F) was calculated using diclazuril sodium salt as a reference.*

The pharmacokinetic parameters of diclazuril sodium salt after administration as a feed additive formulation are shown in Table 4. Analysis of the plasma samples showed rapid absorption of diclazuril after administration of diclazuril sodium salt as a feed additive (Figure 6). Observed peak plasma concentrations of diclazuril were obtained within 4 to 24 hours after administration and ranged from a low of 2,000 ng/ml to a high of 3,200 ng/ml. The mean observed peak plasma concentration of diclazuril was 2,500 (±558 SD) ng/ml and was obtained at 8 hours after administration (data not shown). Thereafter, observed plasma concentration declined to 345 (±80 SD) ng/ml at 168 hours after administration, with an apparent average elimination half-life of 54 hours. The relative oral bioavailabilities of diclazuril sodium salt as a feed additive compared with

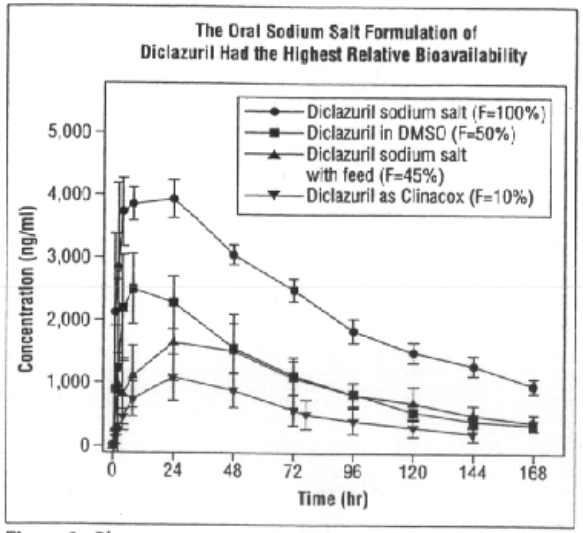

Figure 8. *Plasma concentrations and relative oral bioavailability (F) of various diclazuril formulations following oral administration in horses. Relative bioavailability was calculated using oral-mucosal administration of diclazuril sodium salt as a reference.*

oral-mucosal administration ranged from a low of 34% to a high of 54%, with the mean oral bioavailability of diclazuril sodium salt as a feed additive being 45% (Figure 7).

Figure 8 provides a comparison of the mean plasma concentrations of diclazuril following oral administration of diclazuril as Clinacox (5 mg/kg),[11] oral administration of diclazuril in DMSO (2.2 mg/kg), oral-mucosal administration of diclazuril sodium salt (2.2 mg/kg), and oral administration of diclazuril sodium salt as a feed additive. The bioavailability of diclazuril as Clinacox ranged from 5% to 14% relative to oral-mucosal administrations of diclazuril sodium salt, with a mean bioavailability of 10%. Relative bioavailability of diclazuril in DMSO compared with oral-mucosal administration of diclazuril sodium salt was 50%, indicating approximately half the bioavailability of diclazuril in DMSO following oral administration. Additionally, the relative oral bioavailability of diclazuril sodium salt as a feed additive compared with oral-mucosal administration of diclazuril sodium salt was 45%.

■ DISCUSSION AND CONCLUSION

In earlier studies, many of us along with other researchers identified triazine-based antiprotozoal agents as potentially important therapeutic agents for use in the treatment of EPM.[6,10,11] Triazine-based antiprotozoal agents have lipophilic characteristics, which may facilitate absorption following oral administration. Additionally, absorption of compounds from the gastrointestinal (GI) tract depends on the physiochemical properties of the compound, such as lipid solubility and dissociation rate.[15] It is often generalized that an increase in lipid solubility increases the absorption of a drug from the GI tract. However, extremely hydrophobic compounds, such as triazine antiprotozoal drugs, have low solubility in GI fluids, which results in low absorption and bioavailability.[15] If the compound is a solid and is relatively insoluble in GI fluids, it will have limited contact with the GI mucosa, and therefore, its rate of absorption will be low.

Bioavailability is an important parameter in clinical trials because most of a drug's therapeutic and toxicologic effects are proportional to both dose and bioavailability. Additionally, poor oral bioavailability results in variable and poorly controlled plasma concentrations and drug effects. It is therefore important to maximize oral bioavailability of triazine-based agents with the goal of maximizing the ability

to control plasma drug concentrations and thus the clinical efficacy of these agents.

In a previous study,[11] it was suggested that the oral bioavailability of triazine-based antiprotozoal agents may vary among individual horses in a clinically significant manner. Three possible solutions to deal with highly variable oral bioavailability of triazine-based antiprotozoal agents were proposed.[11] The most practical was the development of a formulation that would provide greater oral bioavailability. The results of this study show that the sodium salt formulation of diclazuril is well absorbed following oral administration and has the potential to be used as a feed additive.

It is known that the best way to determine the comparative bioavailability of different drug formulations is to use a Latin square design (crossover) by comparing each animal to itself as a control following appropriate washout periods. Unfortunately, we did not have all these diclazuril formulations when we started working with triazine agents, including pure diclazuril, and therefore, in this study, four different groups of four horses were dosed orally with different formulations of diclazuril at different times. It should be remembered that the main point of this study was not to determine the exact magnitude of the relative bioavailability of different formulations of diclazuril but simply to show that there are large, potentially clinically significant differences in terms of absorption following oral administration of different formulations of diclazuril. It should be remembered that one of the disadvantages of Latin square design is the washout period, and in this model we assumed that systemic clearance does not change in each study subject during the waiting period. Additionally, since the plasma concentrations of diclazuril were relatively closely distributed among horses in each study group, we strongly believe that we would not see a drastically different result if the same horses were crossed over by using different oral formulations of diclazuril.

In conclusion, there is substantial preliminary evidence that sodium salt formulations of diclazuril can be expected to:

- Increase the therapeutic and prophylactic efficacy of a given dose of the active agent compared with current formulations
- Improve dosing characteristics by reducing inter- and intrasubject variability in treatment response groups as a result of very poor and highly variable absorptions of current formulations
- Reduce potential development of drug-resistant strains of disease as a result of survival, adaptation, and selection among parasites in undertreated subjects having lower absorption rates of existing diclazuril treatments
- Improve the ability to show clinical trial efficacy against a broader range of protozoan-mediated diseases
- Enable development of species-specific, easily administered pharmaceutical products, including feed additives, for treatment and prophylaxis of EPM and various other apicomplexan-mediated diseases.

The findings presented here indicate that additional studies on the use of these compounds for treatment of EPM are warranted.

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