

Diclazuril in the horse: its identification and detection and preliminary pharmacokinetics

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Publication #242 from the Equine Pharmacology and Experimental Therapeutics Program at The Maxwell H. Gluck Equine Research Center and the Department of Veterinary Science, University of Kentucky
Published as Kentucky Agricultural Experiment Station Article # 98-14-70 with approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station

Dirikolu, L., Lehner, F., Natrass, C., Bentz, B. G., Woods, W. E., Carter, W. G., Karpiesiuk, W., Jacobs, J., Boyles, J., Harkins, J. D., Granstrom, D. E., Tobin, T. Diclazuril in the horse: its identification and detection and preliminary pharmacokinetics. *J. vet. Pharmacol. Therap.* 22, 374–379.

Diclazuril (4-chlorophenyl [2,6-dichloro-4-(4,5-dihydro-3H-3,5-dioxo-1,2,4-triazin-2-yl)phenyl] acetonitrile), is a benzeneacetonitrile antiprotozoal agent (Janssen Research Compound R 64433) marketed as Clinacox[®]. Diclazuril may have clinical application in the treatment of Equine Protozoal Myeloencephalitis (EPM). To evaluate its bioavailability and preliminary pharmacokinetics in the horse we developed a sensitive quantitative high-pressure liquid chromatography (HPLC) method for diclazuril in equine biological fluids. MS/MS analysis of diclazuril in our HPLC solvent yielded mass spectral data consistent with the presence of diclazuril.

After a single oral dose of diclazuril at 2.5 g/450 kg (as 500 g Clinacox[®]), plasma samples from four horses showed good plasma concentrations of diclazuril which peaked at $1.077 \pm 0.174 \mu\text{g/mL}$ (mean \pm SEM) with an apparent plasma half-life of about 43 h. When this dose of Clinacox[®] was administered daily for 21 days to two horses, mean steady state plasma concentrations of 7–9 $\mu\text{g/mL}$ were attained. Steady-state levels in the CSF ranged between 100 and 250 ng/mL. There was no detectable parent diclazuril in the urine samples of dosed horses by HPLC or by routine postrace thin layer chromatography (TLC).

These results show that diclazuril is absorbed after oral administration and attains steady-state concentrations in plasma and CSF. The steady state concentrations attained in CSF are more than sufficient to interfere with *Sarcocystis neurona*, whose proliferation is reportedly 95% inhibited by concentrations of diclazuril as low as 1 ng/mL. These results are therefore entirely consistent with and support the reported clinical efficacy of diclazuril in the treatment of clinical cases of EPM.

(Paper received 11 September 1998; accepted for publication 7 September 1999)

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INTRODUCTION

Equine Protozoal Myeloencephalitis (EPM) is a chronic infectious neurological disease of American horses caused by the parasitic apicomplexan *Sarcocystis neurona*. Current treatments for EPM include administration of pyrimethamine/sulfonamide combinations. Although treatment with these agents is successful in many cases, treatment can be prolonged and resistance to treatment or relapse after cessation of treatment is not uncommon (Granstrom *et al.*, 1997).

Diclazuril is a benzeneacetonitrile anticoccidial with proven efficacy against intestinal *Eimeria* species in avian, intestinal and hepatic coccidiosis in rabbits, and against toxoplasmosis in mice (Lindsay *et al.*, 1995). Diclazuril has also been evaluated for treatment of isosporiasis and cryptosporidiosis in AIDS patients. Diclazuril may be selectively toxic for apicomplexans

(Hackstein *et al.*, 1995); it has been shown that apicomplexan parasites contain a plastid-like chloroplast organelle for binding of triazine-based antiprotozoal agents and it is believed that apicomplexa acquired this plastid by secondary endosymbiosis, probably from a green alga (Kohler *et al.*, 1997). Diclazuril has been suggested to have potential activity against *S. neurona* (Granstrom *et al.*, 1997) and preliminary data suggest that it may have clinical application in the treatment of EPM (Bentz *et al.*, 1998). In recent study, the efficacy of diclazuril in inhibiting merozoite production of *S. neurona* and *S. falcatula* in bovine turbinate cell cultures was studied (Lindsay & Dubey, 1999). This study has shown the inhibition of merozoite production by more than 80% in cultures of *S. neurona* or *S. falcatula* treated with 0.0001 $\mu\text{g/mL}$ diclazuril and greater than 95% inhibition of merozoite production with 0.001 $\mu\text{g/mL}$ diclazuril.

This pilot study describes the development and characterization of a quantitative HPLC method for diclazuril in the biological fluids of horses and also includes a preliminary evaluation of the pharmacokinetics of this agent in the horse. Because diclazuril may be used in performance horses, we also performed a preliminary evaluation of its detectability in equine urine by HPLC and TLC as well as the efficacy of mass spectrometric identification of this material.

MATERIALS AND METHODS

Horses and sample collection

Four mature Thoroughbred mares weighing 461–576 kg were used for this study. Diclazuril was obtained in milligram amounts as the authentic standard along with the recommended internal standard from Janssen Pharmaceuticals (Beerse, Belgium). For administration to horses the only form of diclazuril available to us was Clinacox[®], a poultry feed premix imported from Pharmacia-Upjohn (Ontario, Canada). Clinacox[®] is 0.5% diclazuril, 99.5% protein carrier. It was administered to horses at a single dose of 5 mg/kg of diclazuril suspended in 6–8 L of water, by nasogastric intubation. Blood samples were collected into heparinized tubes that were centrifuged at 4 °C 2000 × *g* for 15 min, and the plasma stored at –20 °C until assayed.

In a second experiment, diclazuril (5 mg/kg body weight as Clinacox[®]) was administered daily for 21 days to two horses with a presumptive diagnosis of EPM. Blood samples were collected as described above. Urine collection was accomplished with a Foley catheter and attached plastic bag. Urine was placed in aliquots and stored at –20 °C until assayed. Cerebrospinal fluid (CSF) samples were routinely collected at the lumbosacral space by a licensed veterinarian (B.G.B.). The cerebrospinal fluid was retained for analysis if there was no visible evidence of blood contamination and stored in serum tubes at –20 °C until analysed. All animal care was in compliance with the guidelines issued by the Division of Laboratory Animal Resources and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky.

Tandem quadrupole mass spectrometric analysis (MS/MS)

MS/MS was performed to evaluate the efficacy of this technique as a potential HPLC detector for diclazuril. A solution of diclazuril 10 µg/mL in HPLC mobile phase (see HPLC section below) was infused at 10 µL/min into the electrospray (ES) ionization probe of a Micromass Quattro II tandem quadrupole mass spectrometer (MS (Micromass, Beverly, MA, USA)). Daughter ion analysis in the second quadrupole was achieved with collision induced dissociation (CID) using argon as collision gas at 2.610^{–4} mBar. Both positive and negative ion modes were attempted. Positive mode ES was calibrated with a solution of mixed polypropylene glycols, whereas negative mode was calibrated with 3 µg/mL NaF and 0.5 µg/mL NaI in 50:50 isopropanol:water. Other conditions are described in the text.

Diclazuril by high pressure liquid chromatography (HPLC)

Sample preparation

A standard solution of 1 mg diclazuril (Janssen R 64433) was prepared in 1 mL HPLC grade N, N-dimethylformamide [DMF (Sigma-Aldrich, St. Louis, MO 27,054–7)]. Standards were prepared by the addition of a specified amount of diclazuril in 50% DMF/50% water to blank plasma samples, 1 mL each, over a range from 0.25 to 10 µg/mL. Janssen compound R 62646, a structural analogue of diclazuril, was used as the internal standard. The internal standard was prepared in 1 mL DMF (1 mg/mL) and diluted 1 to 10 in 50% DMF/50% water to yield 100 ng/µL standard solution. To each sample, 20 µL of 100 ng/µL internal standard was added. Then, 2 mL of 0.1 M phosphate buffer (pH 6.0) was added to each sample and the pH was adjusted to 6.0 as necessary.

Extraction method

Analytichem C-18 'Mega Bond Elut' columns (Varian, Harbor City, CA) were placed into an SPS24 VacElut vacuum chamber and treated sequentially with 2 mL of HPLC grade methanol and 2 mL of 0.1 M phosphate buffer (pH 6.0). The vacuum was turned off as soon as buffer reached the top of the sorbent bed to prevent column drying. The specimen was drawn slowly through the column taking at least 2 min to pass specimen through the Mega Bond Elut column. The column was then rinsed sequentially with 2 mL of 0.1 M 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 5–10 min after each rinse. A labelled silanized glass tube was placed below the column and an eluate was collected by slowly rinsing the column with 4 mL of methanol:HCl (conc), 95:5. The solvent was evaporated under a stream of nitrogen gas at 40 °C using silanized taper bottom tubes. The residue was resuspended first in 100 µL of DMF with moderately vigorous vortexing and sonication. After that, 100 µL of water was added, and the resuspension procedure was repeated, resulting in a 50:50 (DMF:water) solution. This solution was placed into a 300 µL vial for HPLC analysis.

Instrumentation

The HPLC procedure was adapted from that described by Kock *et al.* (1992). The instrument employed was a Beckman System Gold HPLC system with two 110B solvent delivery pumps, a 168 photodiode array detector and a 502 autosampler. The column was a Beckman Ultrasphere ODS, 5 mm particle size, 4.6 mm × 15 cm column size, protected with an Altech C-18 guard column. The mobile phase consisted of 46% solvent A and 54% solvent B run with a flow rate of 1 mL/min. Solvent A was (80% [0.5% ammonium acetate, 0.01 M tetrabutylammonium hydrogen sulfate (TBAHS) (Sigma # 39684–2) in water]: 20% acetonitrile). Solvent B was (80% methanol, 20% acetonitrile). Acetonitrile (A998–4, Fisher Scientific, Fair Lawn, NJ) and methanol (MX0488–1, EM Science, Gibbstown, NJ) were HPLC grade. After preparation, solvents A and B were degassed and filtered with 0.45 µm type HV Millipore filters. The diode array detector was set up for single wavelength acquisition at 280 nm with a 12 nm span. Twenty µL injections were made with a 20 µL loop.

Urine analysis by thin layer chromatography (TLC)

Urine samples were analysed by routine postrace testing techniques as used in Kentucky postrace testing. The following standard National Association of State Racing Commissioners (NASRC) Quality Assurance Program (1982–88)/Association of Racing Commissioners International (ARCI) Quality Assurance Program (1988–95) (personal communication from Truesdail Laboratory) methods were utilized in an attempt to detect diclazuril:base hydrolysis with acid extraction (BH), the neutral urine extraction (NU), enzyme hydrolysis with alkaline extraction (EH), ion pair urine extraction (IPU), and base urine extraction (BU) techniques were employed as well as six different TLC development solvents in an attempt to establish whether any of these systems were capable of identifying diclazuril or its metabolites.

RESULTS

Tandem quadrupole mass spectrometry by negative mode electrospray was performed on diclazuril dissolved in our HPLC mobile phase. Diclazuril was not evident by positive mode electrospray at any pH in the 3–7 range. Interpretable spectra were obtained with ES-negative mode verifying the integrity of the diclazuril standard and demonstrating its negative charge in

solution (Fig. 1). However, the overwhelming intensity of the ion pair reagent TBAHS and its capacity to complex with negatively charged species to form multiple aggregates in our mobile phase convinced us to consider UV diode array detection as our most sensitive alternative for routine HPLC detection and quantification of this agent.

The HPLC diode array detection method reported here readily detects diclazuril in plasma, with a limit of detection of diclazuril in plasma of about 10 ng/mL. Satisfactory recovery (82%) was obtained for solid phase extraction of diclazuril from plasma samples of horses on HPLC. The diclazuril peak eluted at around 13.00 min (± 0.8 min), and the internal standard peak eluted at 14.50 min (± 0.8 min) (Fig. 2). The peaks were symmetrical and the standard curve was linear from 0.25 to 10 $\mu\text{g/mL}$ with an r -value 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.

After administration of single oral doses of diclazuril (as Clinacox[®]) to four horses, analysis of plasma samples showed significant plasma concentrations (Fig. 3), with a mean (SEM) peak plasma concentration of $1.077 \pm 0.174 \mu\text{g/mL}$ of diclazuril

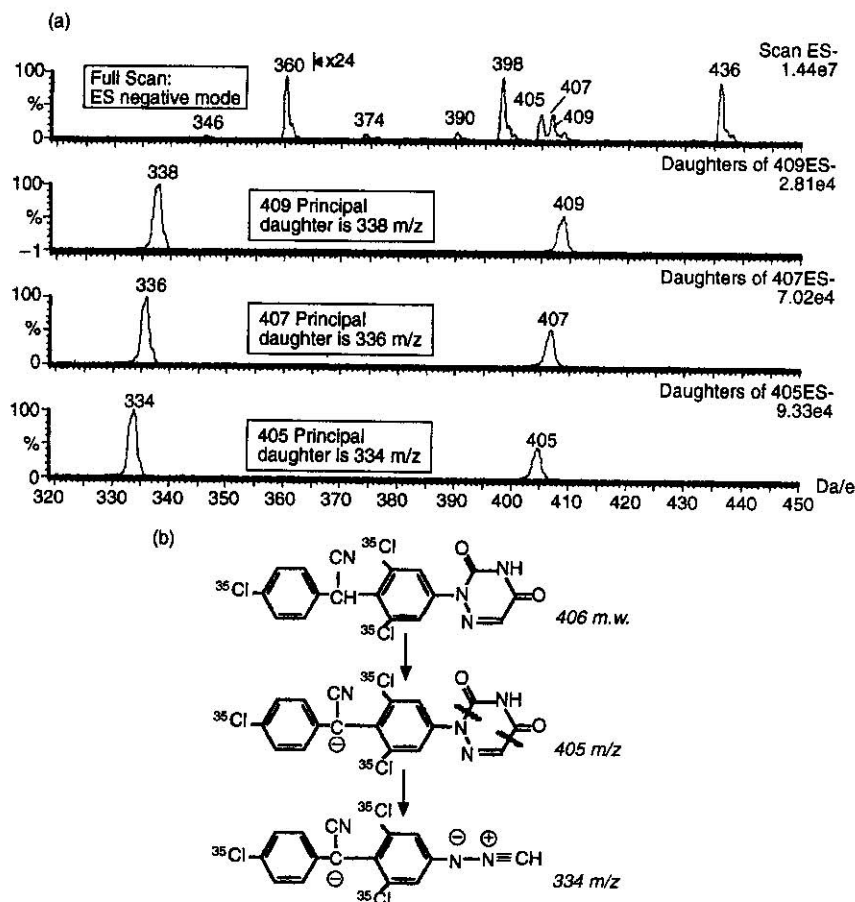


Fig. 1. Negative mode electrospray ionization of diclazuril in HPLC mobile phase for assessment of tandem quadrupole MS as a detector. (a) Continuum mode mass spectra. Full scan spectrum in uppermost panel: principal ions 360, 398, 436 m/z represent ion pair reagent tetrabutylamine complexes. The triplet 409, 407, 405 m/z represent $^{35}\text{Cl} \ ^{37}\text{Cl}_2$, $^{35}\text{Cl}_2 \ ^{37}\text{Cl}$, and $^{35}\text{Cl}_3$ versions of diclazuril minus a proton, respectively. Daughter ions of 409, 407, and 405 m/z are shown in the lower three panels. Collisionally induced dissociation (CID) of diclazuril-specific species gave single prominent peaks representing loss of 71 amu. (b) Hypothetical scheme to account for mass values during principal diclazuril CID, with fragmentation of the triazidone ring and loss of $\text{O}=\text{C}-\text{NH}-\text{C}=\text{O}$, shown here for the 405 m/z $^{35}\text{Cl}_3$ anion. CID was induced with Argon gas (2.6×10^{-4} mbar), collision energy = 33, cone voltage = 26.

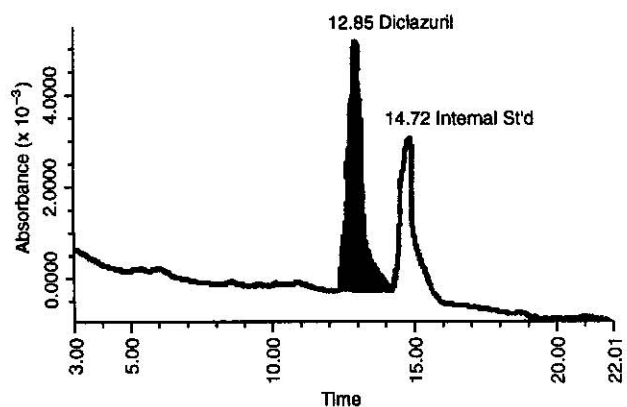


Fig. 2. A typical HPLC chromatogram of diclazuril with internal standard (methylated diclazuril) in dimethylformamide:water, 50:50 as extracted from dosed horse plasma sample. Absorbance at 280 nm is plotted vs. retention time. The instrument is a Beckman System Gold HPLC system with Beckman ODS column 5 μ particle size, 4.6 mm \times 15 cm column size. The mobile phase consisted of 46% solvent A (80% (0.5% ammonium acetate, 0.01 M tetrabutylammonium hydrogen sulfate in water): 20% acetonitrile) and 54% solvent B (80% methanol, and 20% acetonitrile) at a flow rate of 1 mL/min. The photodiode array detector wavelength was set at 280 nm.

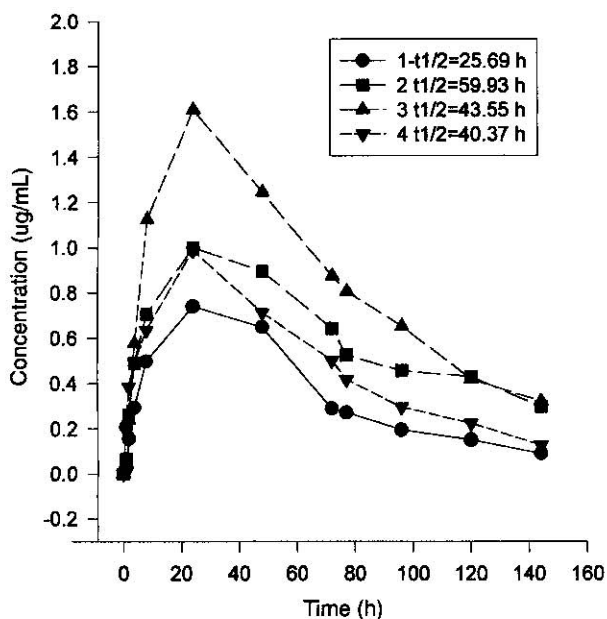


Fig. 3. Plasma concentrations of diclazuril from four horses following single p.o. dose of diclazuril at 2.5 g/450 kg (as Clinacox[®]). Legend includes individual plasma half-lives as estimated by PK Analyst software.

at 24 h after administration. Thereafter, the plasma concentration declined to 208 ± 58 ng/mL at 144 h after administration with an apparent average half-life of ~ 43 h.

Daily administration of Clinacox[®] (5 mg/kg diclazuril) to two horses yielded steady state plasma concentrations of diclazuril of 7–9 μ g/mL after approximately 6–8 days (Fig. 4). Steady state concentrations of diclazuril in the cerebrospinal fluid samples ranged between 100 and 250 ng/mL (Fig. 5). No detectable

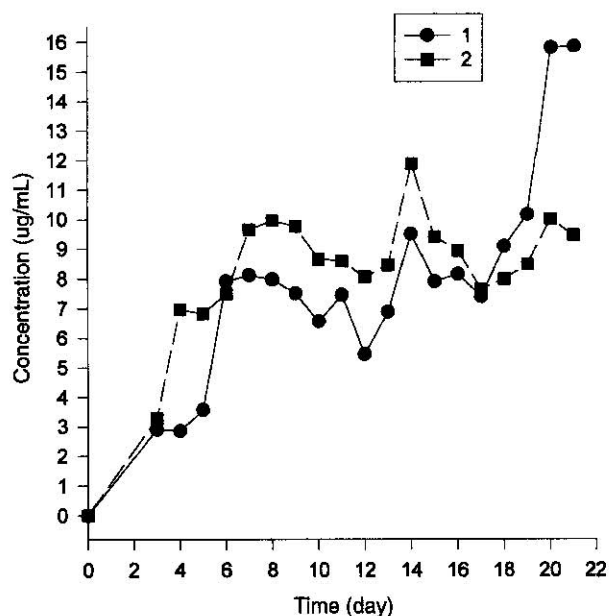


Fig. 4. Plasma concentrations and steady state plasma concentrations of diclazuril from two horses following daily oral administration of 2.5 g/450 kg diclazuril as Clinacox.

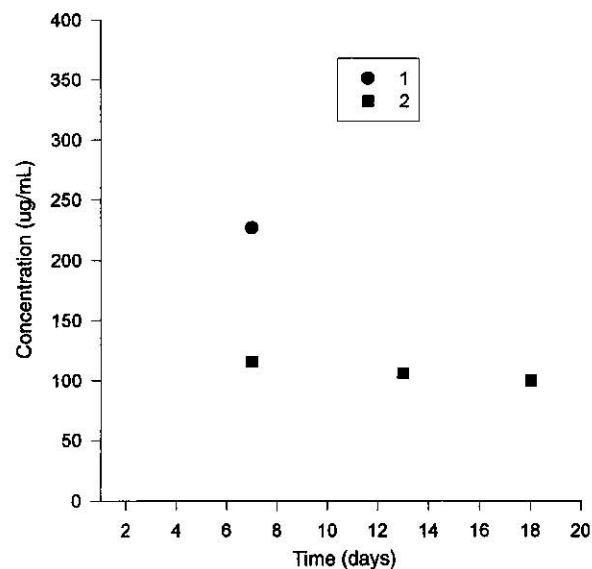


Fig. 5. Cerebrospinal fluid concentrations of diclazuril at steady state plasma concentrations of diclazuril from two horses following daily oral administration of diclazuril at 2.5 g/450 kg as Clinacox.

concentration of parent diclazuril was identified in any urine samples using this HPLC methodology.

For TLC analysis, standard diclazuril was spotted on the TLC plates, along with the urine residues. Parent diclazuril yielded a faint reaction with Dragendorff's reagent followed by cupric chloride and sodium nitrite overspray. None of the described TLC methods were able to discern spots in post administration samples that could be specifically associated with diclazuril, whether as parent compound or as metabolite.

DISCUSSION

We had earlier identified diclazuril as a potentially important therapeutic agent for use in the treatment of Equine Protozoal Myelitis (EPM) (Granstrom *et al.*, 1997; Bentz *et al.*, 1998); as such we needed a sensitive and reliable analytical method to perform preliminary bioavailability and pharmacokinetic experiments with this agent in the horse. In particular, the method selected needed to be sufficiently sensitive to detect low concentrations of parent diclazuril in equine plasma, urine and cerebrospinal fluid samples from test horses.

We elected to use an HPLC approach, under which condition diclazuril was likely to be considerably more stable than during exposure to the high temperatures required by GC/MS. To establish the stability of diclazuril under such conditions we dissolved diclazuril in the initial HPLC mobile phase and introduced the resultant solution directly into our MS/MS. Diclazuril could be visualized only by negative ion MS/MS, which revealed its presence as a deprotonated anion. Figure 1 shows the occurrence of a single prominent daughter ion from each of the chlorine isotopic variants of diclazuril, in each case representing the loss of 71 atomic mass units. In addition to diclazuril, Fig. 1 shows that the ion pair reagent TBAHS forms numerous aggregates with negatively charged species possibly suppressing the signal obtainable from diclazuril-specific ions. The TBAHS is thus advantageous for reverse phase chromatography, but restricts the applicability of MS/MS as a suitable means of quantitation. Direct infusion MS/MS is nevertheless a suitable means of qualitative identification of diclazuril in biological extracts.

Based on these observations we elected to continue with the development of our HPLC-UV diode array approach for the detection of diclazuril in equine plasma and CSF samples. The resultant HPLC method is a sensitive analytical method with the limit of detection for diclazuril being about 10 ng/mL from extracted plasma samples. Additionally, satisfactory recovery (82%) was obtained for diclazuril from plasma samples. The chromatograms of both diclazuril and its internal standard are symmetrical, demonstrated good linearity and more than sufficient sensitivity to detect diclazuril in equine plasma and cerebrospinal fluid.

When diclazuril was administered to four horses at a dose of 2.5 g/450 kg. by stomach tube as Clinacox[®], this drug was detectable in plasma within one hour, after which the plasma concentrations increased to a peak at about 24 h post dosing. The mean plasma concentrations of diclazuril in the four horses tested in these preliminary experiments were relatively closely distributed, with the range of the peak concentrations falling between 0.75 and 1.6 µg/mL at 24 h post dosing. Thereafter the plasma concentrations of diclazuril declined to less than 0.5 µg/mL by 148 h post administration, but diclazuril was still readily detectable in the plasma of each horse at 148 h post dosing. Similarly, the apparent plasma half-lives of diclazuril in each horse varied from a low of 26 h to a high of 59 h with the average half-life of diclazuril being 43 h.

Because sufficient pure parent diclazuril was unavailable to us, we were unable to repeat these oral administrations of

Clinacox[®] with parallel intravenous (i.v.) administrations. In the absence of such data we are unable to rigorously estimate the oral bioavailability of diclazuril as Clinacox[®] administered to these horses. Nevertheless, what data exist show that diclazuril is reasonably well absorbed after oral administration as Clinacox[®] to horses, and that this drug has a relatively long plasma half-life (~43 h) following administration by this route. The data also suggest that repeated oral administration of diclazuril will give rise to useful steady state plasma concentrations of this drug, although variability in the final steady state concentrations between individual horses may be expected. However, these individual and well-maintained steady-state plasma concentrations of diclazuril should enable diclazuril to enter the central nervous system of horses and achieve steady state concentrations in the cerebrospinal fluid. We therefore selected two horses for treatment with oral diclazuril, administered as Clinacox[®], 500 g/day (2.5 g diclazuril/day) for 21 days.

As shown in Fig. 4, daily administration of diclazuril to two horses at 2.5 g/day as 500 g of Clinacox[®] for 21 days yielded steady state plasma concentrations of 7–9 µg/mL after approximately 8 days and these plasma concentrations were well maintained throughout the duration of therapy. Diclazuril was also detected in cerebrospinal fluid of these horses, although at significantly lower concentrations than were observed in plasma, namely between 100 and 250 ng/mL. These data show that diclazuril enters the CSF from plasma and is maintained there. One possible interpretation for the lower CSF concentrations of diclazuril is that diclazuril may be highly plasma protein bound. In any event, the presence of free diclazuril in the CNS at even these relatively low free concentrations is consistent with reports of the observed therapeutic efficacy of diclazuril in the treatment of Equine Protozoal Myelitis (EPM) (Granstrom *et al.*, 1997; Bentz *et al.*, 1998), because it appears that *S. neurona* is exceptionally sensitive to diclazuril.

It should be borne in mind that even these preliminary experiments suggest that the oral bioavailability of Clinacox[®] may vary between individual horses in a clinically significant manner. For example, in our small sample of four horses there was a two fold difference between the peak plasma concentrations of diclazuril observed in the high (1.6 µg/mL) and the low (0.75 µg/mL) horses. These differences presumably translate into equivalent differences in steady-state concentrations of diclazuril attained in plasma and ultimately in the CSF of treated animals.

These apparent differences in the bioavailability of Clinacox[®] may represent a possible source of apparent therapeutic failure with diclazuril or, more likely, a possible source of short-term 'relapses' after completion of a course of therapy with this agent. This is because in any large series of experimental animals there will be some individual animals that will have difficulty in absorbing sufficient diclazuril from Clinacox[®] to yield fully effective plasma and CSF therapeutic concentrations of this agent.

Two possible solutions to this problem immediately present themselves. The simplest is the development of a highly bioavailable preparation of diclazuril which will routinely yield effective plasma and CSF concentrations of this agent in all horses treated. A secondary approach is to monitor plasma concentrations of

diclazuril during therapy and adjust the oral dose to compensate for any deficits in bioavailability in individual animals.

Finally, EPM is not uncommon in horses in training and also in racing, and some horses in training are routinely maintained on pyrimethamine/sulfonamide treatment for EPM throughout their racing careers. We therefore looked for evidence of the presence of diclazuril or its metabolites in urine from these horses to determine the likelihood of these horses showing evidence for the presence of diclazuril or its metabolites during routine postrace testing. To our considerable surprise we found no evidence for the presence of diclazuril or any potential metabolites or breakdown products in these postadministration urines by our standard HPLC method for diclazuril. In addition, postadministration urine samples sent to Truesdail Laboratories for routine postrace screening showed no evidence for the presence of diclazuril or its metabolites in any postadministration urine. This lack of detectable levels of diclazuril or potential breakdown products in equine urine samples with either HPLC or TLC suggests that diclazuril is either highly lipophilic or highly metabolized in the horse or both. Clearly, further studies are required to determine the metabolic fate of diclazuril in the horse as well as its pharmacokinetics and disposition.

These results are consistent with the apparent clinical efficacy of diclazuril in the treatment of EPM. Specific disposition and pharmacokinetic studies, further research on its clinical efficacy, and the defining of the appropriate therapeutic window for diclazuril as a treatment for EPM in the horse require further study. Above all, a more suitable and appropriate formulation of this agent for administration to horses other than Clinacox[®] is required, as Clinacox[®] is a poultry feed which is a less than optimal equine formulation.

ACKNOWLEDGMENTS

This research was supported by grants from the United States Trotting Association, Claiborne Farms, American Live Stock

Insurance, Three Chimneys Syndicates, Juddmonte Farms, the Kentucky Thoroughbred Farms Manager's Club, Castleton Farm, The Deputy Minister Syndicate, the Forest Wildcat Syndicate, Rood & Riddle Equine Hospital, HagyardDavidson-McGee Veterinary Practice, Walnut Hill Limited, W. Bruce Lunsford, Mrs. Adele B. Dilschneider, Darley Stud Management Inc., Ogden Mills Phipps, Ogden Phipps, Edward A. Cox, and Cherry Valley Farm.

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Molecular and pharmacological characterization of the canine brainstem alpha-2A adrenergic receptor

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Schwartz, D. D., Jones,

W. G., Hedden, K. P., Clark, T. P. Molecular and pharmacological characterization of the canine brainstem alpha-2A adrenergic receptor. *J. vet. Pharmacol. Therap.* 22, 380–386.

This study characterizes the α_2 -adrenergic receptors present in canine brainstem. Radioligand binding and reverse transcriptase-polymerase chain reaction (RT-PCR) experiments were performed in canine brainstem to identify the receptors present and determine the pharmacological properties of these receptors. The pK_i values derived from radioligand competition curves for a number of adrenergic receptor agents at the four α_2 -adrenergic receptor subtypes were compared to the canine brainstem. The pK_i values at the canine brainstem α_2 -adrenergic receptor were consistent with the presence of the α_{2A} -adrenergic receptor. To determine whether the canine brainstem expressed the message for the α_{2A} -adrenergic receptor, RT-PCR was performed with specific primers for the four subtypes of α_2 -adrenergic receptors. In the canine brainstem, only the primers corresponding to a region in the human α_{2A} -adrenergic receptor produced a PCR product. No bands were detected in the canine brainstem lanes with the α_{2B} -, α_{2C} -, or α_{2D} -receptor primers. These data suggest that the canine brainstem contains the α_{2A} -adrenergic receptor.

(Paper received 18 February 1999; accepted for publication 7 September 1999)

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INTRODUCTION

The role of central α_2 -adrenergic receptors in the regulation of cardiovascular function, sedation and analgesia is well established (Ruffolo *et al.*, 1991; Ruffolo & Hieble, 1994). The centrally mediated sedative and analgesic properties of α_2 -adrenergic receptor agonists have found prominent clinical applications in veterinary medicine and are used extensively in dogs. α_2 -Adrenergic receptors are comprised of numerous subtypes based on classical pharmacology and molecular biology studies (Bylund, 1988; Aantaa *et al.*, 1995) and are distributed throughout the central nervous system in a nonhomogeneous manner (Scheinin *et al.*, 1994; MacDonald & Scheinin, 1995).

There are now three well characterized α_2 -adrenergic receptor subtypes: α_{2A} -, α_{2B} - and α_{2C} - (Harrison *et al.*, 1991; Aantaa *et al.*, 1995; MacDonald *et al.*, 1997). A fourth subtype, α_{2D} -, represents the rodent homologue of the α_{2A} -adrenergic receptor (Blaxall *et al.*, 1993). Pharmacologically, the α_{2A} - and α_{2B} -adrenergic receptors were originally distinguished from each other based on their relative affinities for oxymetazoline and prazosin. The α_{2A} -receptor, found in human platelets and HT-29 cells, has a high affinity for oxymetazoline and a low affinity for prazosin, whereas the α_{2B} -adrenergic receptor, found in neonatal rat lung, has a low affinity for oxymetazoline and a high affinity for prazosin. The α_{2C} -adrenergic receptor identified in the

opossum kidney and the OK cell line has an intermediate affinity for oxymetazoline and prazosin. The α_{2D} -adrenergic receptor subtype (Lanier *et al.*, 1991) in bovine pineal and rat submaxillary glands is generally considered to be a species homologue of the human α_{2A} -adrenergic receptor. However, the α_{2D} -adrenergic receptor has a lower affinity for raulwolscine, yohimbine and SKF 104078 compared to the α_{2A} -adrenergic receptor (Blaxall *et al.*, 1993).

The genes for the three human α_2 -adrenergic receptor subtypes have been cloned and are designated α_2 -C10, α_2 -C2 and α_2 -C4 and correspond to the α_{2A} -, α_{2B} - and α_{2C} -subtypes, respectively (Harrison *et al.*, 1991; Bylund *et al.*, 1992). The gene for the α_{2D} -adrenergic receptor (RG20) has also been cloned (Lanier *et al.*, 1988) and shares considerable sequence homology with the α_{2A} -adrenergic receptor despite the difference in ligand binding characteristics (Blaxall *et al.*, 1993).

The sedative effects of α_2 -adrenergic receptor agonists are mediated by receptors located primarily on locus coeruleus neurons in the pons of the lower brainstem (Svensson *et al.*, 1975; Cedarbaum & Aghajanian, 1976). In the rat, the locus coeruleus is abundant in the mRNA coding for the α_{2A} -adrenergic receptor (Scheinin *et al.*, 1994). Also, polyclonal antisera directed against a portion of the α_{2A} -adrenergic receptor has been used to show the localization of α_{2A} -adrenergic receptor-like immunoactivity in the brainstem of rats (Rosin *et*