Special Article

Diclazuril and equine protozoal myeloencephalitis (EPM): a clinical report

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Introduction

Equine protozoal myeloencephalitis (EPM) is a nervous system disease caused by the protozoan *Sarcocystis neurona*. The clinical syndrome caused by the disease can have a variety of presentations. Classically, horses present with asymmetric ataxia and weakness defined by multifocal central nervous system lesions. Successful management of this disease has been frustrated by failure of treatment success and by relapse after withdrawal of therapy.

Diclazuril is a triazine derivative used in the prophylaxis of coccidiosis in poultry and experimentally in lagamorphs for coccidiosis (Vanparijs *et al*. 1988, 1989; McDougald *et al*. 1991). It has been evaluated for the treatment of isosporiasis and cryptosporidiosis in AIDS patients (Kayembe *et al*. 1989; Menichetti *et al*. 1991; Limson-Pobre *et al*. 1995) It has also been used, to a limited extent, to treat experimentally induced *Toxoplasma gondii* encephalitis in mice (Lindsay *et al*. 1995). These observations suggest that triazine derivatives may be useful in the treatment of diseases such as EPM that are caused by similar organisms.

The chemical structure of diclazuril is shown with other triazine derivatives in **Figure 1**. The triazine ring is present in all of the compounds; a benzeneacetonitrile group (benzene ring with an acetonitrile group attaching it to the centre ring structure) in all compounds except toltrazuril; and all compounds are correctly termed triazinones due to the presence of the ketone groups on the triazine ring structure (Fleeger 1997).

Recent research utilised oral diclazuril (Clinacox) daily for 3 weeks to treat a horse with severe neurological impairment. This treatment resulted in significant clinical improvement (Granstrom *et al*. 1997). Research found that a closely related compound, toltrazuril, was well absorbed after oral administration to horses (Tobin *et al*. 1997). Recent research suggests that triazines may be selectively toxic for apicomplexans due to a chlorophyll *a* D1 complex that serves as the target site for such agents (Hackstein *et al*. 1995). This site is thought to be conserved in apicomplexans and to be absent in mammals (Hackstein *et al*. 1995). Therefore, triazine derivatives may exhibit selective toxicity to these parasites while causing few or no appreciable side effects in an equine patient.

Based on encouraging preliminary evidence of bioavailability, selective toxicity and clinical efficacy, a clinical investigation of the efficacy of diclazuril for the treatment of EPM was initiated. **The purpose was to**: 1) evaluate the clinical efficacy of this compound in the treatment of EPM; 2) establish an effective daily dosage and treatment duration; 3) estimate the rate of relapse at 6 months; and 4) provide preliminary information on the occurrence of adverse effects associated with treatment.

Materials and methods

Clinical cases of equine protozoal myeloencephalitis (EPM) were solicited from equine practitioners throughout the United States. EPM was diagnosed by neurological evaluation, presence of anti-*Sarcocystis neurona* IgG in cerebrospinal fluid and exclusion of other diseases as necessary according to an examining large animal clinician (internist).

Forty-four horses were included in this investigation. Neurological and diagnostic evaluations were obtained from board-certified large animal internists and were

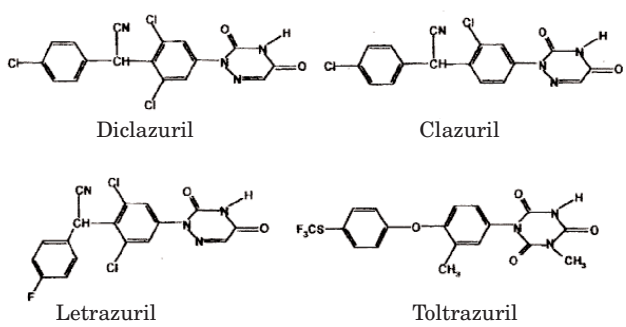

Fig 1: Triazine-based antiprotozoal agents.

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TABLE 1: Breed profile

Breed	No. horses treated
Thoroughbred	23
Paint/Quarter Horse	8
Standardbred	4
Warmblood	3
Tennessee Walker	2
Arabian	1
Rocky Mountain	1
Friesian	1
Missouri Fox Trotter	1

graded on a scale from 0 (normal) to 5 (recumbency due to neurological disease) using a standard neurological examination form. Assessments of neurological grades were subjective and unmasked. A list of diagnostic procedures performed and of differential diagnoses for each case was requested. History, severity of neurological impairment, clinical progression and response to standard therapy were considered in the trial. Preference was given to horses that had been previously diagnosed with and treated for EPM, who exhibited the most severe neurological impairment and who were subjected to the most complete diagnostic evaluations as determined by the first author. Symmetry of neurological deficits was not part of the inclusion criteria. Horses that had not been previously diagnosed with or treated for EPM were considered if their deficits produced a grade 3 or worse and if the case had received thorough diagnostic evaluation.

Follow-up evaluations for both groups were requested at 2 weeks, 6 months and one year after the completion of treatment for each horse. These evaluations included a neurological examination performed by the original examining clinician or internist. They were documented using the same neurological examination form in the initial neurological assessment for inclusion in the investigation. A questionnaire regarding return to work and performance level, worsening of clinical signs during treatment ('treatment crisis'), relapse, degree of clinical change, blood examinations performed, *postmortem* findings, changes in cranial nerve deficits and muscle atrophy was submitted as part of the re-evaluation. The owner, trainer and/or examining clinician completed questionnaires. A repeat CSF immunoblot was requested with the 6 month and 1 year follow-up examinations.

Diclazuril is not currently approved for use in any species in the United States. An Investigational New Animal Drug (INAD) permit was therefore obtained from the Center for Veterinary Medicine at the FDA to allow importation of diclazuril from Canada as a commercially available poultry coccidiocidal premix (Clinacox). Diclazuril was administered *per os* at a dose of 5 mg/kg bwt (500 g Clinacox powder) for 21 consecutive days. Administration of the compound was achieved by addition to the feed. Where palatability precluded complete ingestion of the complete dose, either daily nasogastric intubation was performed or a small nasogastric tube was sutured into place. Following relapse of 2 horses, the dose was adjusted to 5.5 mg/kg bwt and the treatment was extended to 28 days. Six of the 44 horses received the adjusted dosage of 5.5 mg/kg bwt for 28 days. Pretreatment of the horses in the study with a nonsteroidal anti-inflammatory drug (NSAID) was recommended for 2 days prior to initiation of the treatment and through the first week. Continued administration of the NSAIDs was left to the discretion of the attending veterinarian. This regimen was recommended to help control any worsening of clinical signs with treatment ('treatment crisis'). A graduated exercise programme was suggested after completion of the treatment interval.

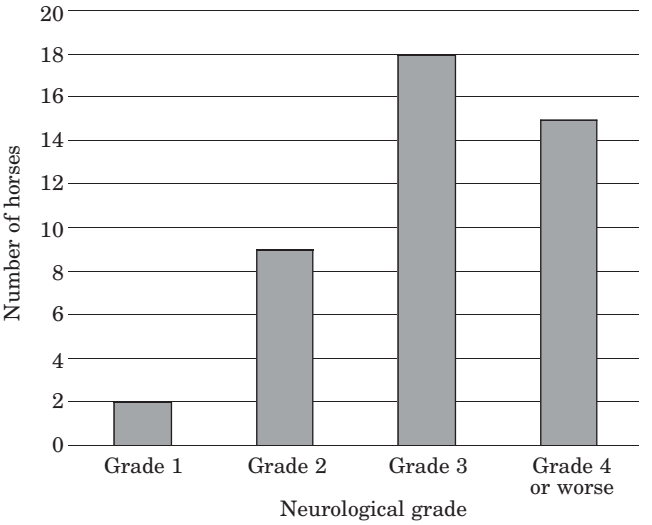

Fig 2: Presenting neurological grades (grade 1–5) (n = 44).

Results

Follow-up information was complete in all 44 cases for only the 6 month post-treatment re-evaluation. Reasons for exclusion from this study included: inadequate information/ unable to rule out other diseases (n = 26); not evaluated by a large animal internist (n = 20); and no follow-up (n = 2). A profile of the breeds of horses is included in **Table 1**. Four horses died or were subjected to euthanasia before treatment was completed. A summary of the presenting neurological grades is presented in **Figure 2**. Thirty-seven of the 44 (84%) cases had been previously diagnosed and treated with standard treatment (pyrimethamine and sulphadiazine) and 7 had not. Thirty-three of the 40 horses that completed the experimental treatment had been previously diagnosed and treated. Thirty-three horses (75%) exhibited asymmetric neurological deficits, 8 horses (18.2%) exhibited cranial nerve deficits, and one horse exhibited seizures. A summary of the diagnostic evaluations performed is presented in **Table 2**.

Six month follow-up examinations were complete for all 40 horses that completed treatment. Follow-up evaluations were obtained from the original examining

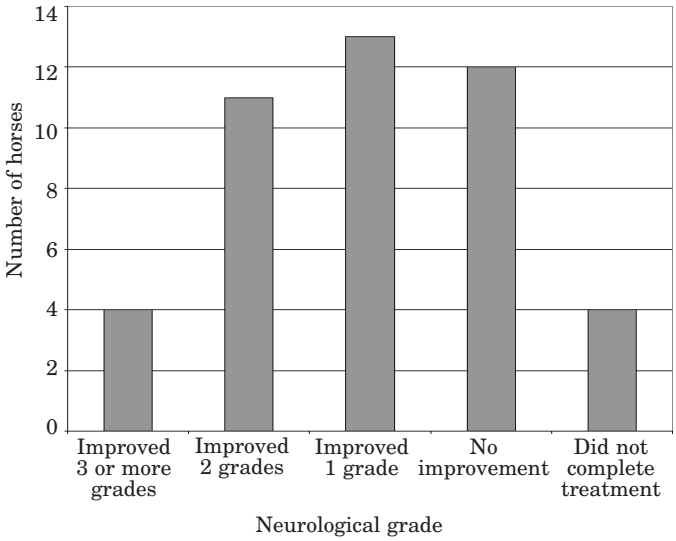

Fig 2: Presenting neurological grades (grade 1–5) (n = 44).

clinician in all but one case. Improvement was reported when re-evaluation of clinical signs using the standard neurological examination form led to a lower grade on the 5 point neurological scale.

Twenty-eight of the 40 horses (70%) that completed treatment were improved. Four of these horses (14.2%) were reported to have improved 3 or more neurological grades. Eleven (39.3%) horses improved 2 to 3 neurological grades. Thirteen horses (46.4%) were reported to improve 1 or 2 neurological grades. Four of the 6 horses receiving 5.5 mg/kg diclazuril for 28 days improved. Twelve of the original 40 (30%) horses failed to improve with treatment. Of 7 horses that had not been previously treated for EPM prior to this investigation, 3 improved (42.9%) and 4 failed to improve (57.1%). Two of the 3 improved horses improved 1 neurological grade, and one horse improved 3 grades. Overall, muscle wasting and cranial nerve deficits appeared to improve more slowly than gait deficits. Six months after the completion of treatment, residual deficits of cranial nerves were reported in 2 horses and muscle atrophy in one horse.

Three of the 4 horses that improved 3 neurological grades were initially *grade 4* or *5* on the neurological scale and one horse was *grade 3* or *4*. Of the 11 horses that improved 2 or 3 neurological grades, 3 horses were initially *grade 4* or *5*, 7 horses were initially *grade 3* or *4* and 1 horse was initially *grade 2* or *3*. Of the 13 horses that improved 1 or 2 neurological grades, 2 horses were initially *grade 4* or *5*, 6 horses were *grade 3* or *4*, 3 horses were *grade 2* or *3*, and 2 horses were initially *grade 1* or *2*.

Statistical analysis was performed using a matched-pairs design using the pre- and post-treatment neurological grades for each horse. A Student's *t* test was performed to determine if there was a significant change in neurological grade from pre- to post-treatment neurological grades. **Figure 3** illustrates the difference in each horse that completed the treatment. The average change in neurological grade was 1.175 towards a lower

TABLE 2: Diagnostics performed (n = 44)

Examination performed	No. horses
Examination by large animal internist	44/44
Serum and CSF immunoblot analysis	43/44*
CSF cytology and protein	37/44
Cervical radiography	31/44
IgG index/albumin quotient	6/44
Myelography	5/44
Skull radiography	3/44
Serum anti-EHV-I titre	2/44
CSF anti-EHV-I titre	2/44
Nerve blocks/lameness exam	2/44
Anti-P2 myelin Ab analysis	1/44
Vitamin E levels	1/44

*CSF aspirate was attempted, but was unable to be obtained safely.

grade. Using a Student's *t* test, the null hypothesis (H_0: that there was no significant difference in neurological grades from pre- to post-treatment grading) was rejected at a α value of 0.025. It is, therefore, reasonable to conclude that there was a statistically significant decrease in the neurological score from pre- to post-treatment.

Adverse effects associated with treatment

Adverse effects reported in association with treatment on the 6 month follow-up questionnaire included: worsening of clinical signs (7/40, 17.5%); colic (1/40, 2.5%); and mild elevation of liver enzymes (AST, GGT) out of reference range (2/40, 5%). Of the 7 horses that exhibited worsening of clinical signs with treatment, 2 were initially *grade 5*, 3 were initially *grade 4*, one was *grade 3* or *4*, and one was *grade 2*. The true occurrence of increase in serum liver enzymes could not be ascertained, as blood examinations were not performed on a majority of the cases included in the investigation.

Relapse

At 6 months after completion of the treatment, relapse was reported in 5.0% (2/40) of the horses that completed treatment. Relapse was defined as worsening of neurological condition by at least one neurological grade for longer than 48 h and after initial clinical improvement. One of the horses that exhibited relapse was suspected to have been underdosed. Underdosing was suspected due to the larger size of the animal and an initial positive response followed by worsening of signs after treatment completion. Retreatment of both horses that relapsed was performed with an adjusted dosage of 5.5 mg/kg bwt for 28 days. This resulted in positive sustained improvement (at least one grade) in both animals. None of the 6 horses that initially received 5.5 mg/kg bwt for 28 days had relapsed 6 months after treatment. Two horses at the Gluck Equine Research Center required euthanasia one year after treatment due to acute recumbency. It is unclear if these horses exhibited relapse or succumbed to their residual neurological deficits. These 2 horses were initially grade 5

and grade 4 on the neurological evaluation and had begun to lose condition. *Postmortem* findings revealed chronic EPM-like lesions in both cases without identification of *S. neurona* or evidence of acute inflammation.

Return to performance

In 27 cases, the initial examining veterinarian, owner or trainer provided information on return to performance with the 6 month evaluation. The types of performance included racing, show, pleasure and breeding animals. The change in the level of performance was evaluated relative to when the horse was believed to be clinically normal. Three of the 27 demonstrated an improved level of performance, 2 of 27 returned to their previous level of performance and 3 of 27 exhibited lower level of performance. Four broodmares were originally treated. Two of these mares were in foal during treatment. These 2 mares subsequently delivered clinically normal foals. Two horses returned to work with no specification on their level of performance. Nine horses were unable to return to work at 6 months after treatment. Of a total of 8 horses that were dead or had been subjected to euthanasia at 6 months after treatment (including the 4 that did not complete treatment), 5 *postmortem* reports were obtained. Two of these horses had lesions compatible with EPM and 2 had lesions indicative of cervical compressive myelopathy (CCM). The aetiology of neurological deficits in one horse was not determined despite *postmortem* examination.

Repeat CSF immunoblots

Re-evaluation of the cerebrospinal fluid by immunoblot analysis was performed in 8 treated horses. Two horses are now CSF negative and 6 horses remain positive. The repeat immunoblots were performed 6–12 months after initial treatment with diclazuril. No other repeat spinal fluid western blots have been reported.

Discussion

Diclazuril is a triazine derivative and is believed to exhibit selective toxicity for apicomplexan parasites. Triazines may be selectively toxic for apicomplexans due to a chlorophyll *a* D1 complex that serves as the target site for such agents (Hackstein *et al*. 1995). Evidence of the presence of chlorophyll *a* bound to the reaction centres of PS II and PS I was identified in trophozoites of *Toxoplasma gondii* (Hackstein *et al*. 1995). These researchers concluded that trophozoites of *Toxoplasma gondii* contain intermediates of chlorophyll biosynthesis and small amounts of chlorophyll *a* bound to the photosynthetic complexes PS I and PS II. A vital component of the photosynthetic reaction centre of PS II is the D1 protein and PS II complexes necessarily contain the D1 protein (Hackstein *et al*. 1995). The *psb*A gene that encodes the D1 protein component of the photosynthetic reaction centre of PS II was amplified from *Sarcocystis muris* cyst merozoites. The PCR sequence appeared from a highly conserved *psb*A gene. The investigators concluded that the deduced amino acid sequence confirmed that the *psbA* gene encodes a D1 protein (Hackstein *et al*. 1995). The putative polypeptide also showed significant amino acid conservation in the putative herbicide-binding region (Hackstein *et al*. 1995). Sensitivity to triazine herbicides has been utilised as direct proof of the vital functions of the chlorophyll *a* D1 complex in apicomplexan parasites (Hackstein *et al*. 1995).

Diclazuril kills apicomplexan parasites by a poorly defined mechanism. One author proposes that diclazuril functions as a nucleotide analogue and is utilised in nucleic acid synthesis. Nuclear growth and division take place in a normal fashion, but later phases of differentiation are inhibited. In general, nucleotide anticoccidials are considered to be lethal and to affect nearly all endogenous development stages (Maes *et al*. 1988; Verheyen *et al*. 1988). Diclazuril is believed to exhibit no antibacterial activity. If diclazuril affords no antibacterial activity and if the chlorophyll complex does function to bind this compound, positive clinical response to the treatment with diclazuril would provide strong support of a diagnosis of EPM. Likewise, lack of response may add support to the presence of an alternative condition.

The initial dose utilised in this investigation (5 mg/kg bwt) was derived from review of literature pertaining to the compound (Lindsay and Blagburn 1994; Hackstein *et al*. 1995; Lindsay *et al*. 1995). This literature suggested coccidiacidal dosages ranging from 1 to 10 mg/kg bwt (Lindsay and Blagburn 1994; Hackstein *et al*. 1995; Lindsay *et al*. 1995). The duration of treatment (3 or 4 weeks in this investigation) was an arbitrary determination that was guided by previous treatment experiences. Early in the investigation, one horse weighing approximately 545 kg was noted to have responded well to the initial dosage and then to have relapsed within 2 weeks of the completion of 21 days of treatment. Pharmacokinetic data for diclazuril became available at this time indicating that full steady state plasma concentrations of this agent were not attained for up to 10 days after initiation of oral administration (Dirikolou *et al*. 1999). Based on this information, the dosage for the investigation was adjusted to 5.5 mg/kg bwt and the treatment interval was extended to 28 days to permit diclazuril to be within the central nervous system for a longer period.

Assessment of clinical response was chosen as the criterion for success of therapy. Treatment duration using standard therapy (pyrimethamine and sulphadiazine) has been advocated until the patient becomes CSF immunoblot-negative (Fenger 1997). Our experience with horses that have received standard treatment suggests that many may remain CSF imunoblot-positive for long periods after successful treatment. The limitation of basing successful outcome on clinical improvement alone is in the subjective nature of the definition of clinical success and the possibility of seeing clinical improvement before all protozoa are eliminated. However, the 6 month rate of relapse was 5.0% in the present investigation. Although the numbers are not directly comparable, this rate

compares favourably to a retrospective study in which EPM was treated by standard therapy. That study reported a 37% occurrence of relapse between 2 weeks and 6 months after discontinuation of treatment in a population of horses treated for various periods (Fenger 1997).

Based on the experiences of the first author, the rate and duration of clinical improvement may be variable. Horses that were treated by the Gluck Equine Research Center were evaluated weekly during treatment, and every 2 weeks thereafter. Evaluations began with the initiation of treatment and were completed 6 months after treatment. All re-evaluations utilised the same protocol as outlined for the initial evaluations. Some horses showed dramatic improvement in as little as one week of treatment. Other horses have appeared to improve on a much more gradual basis. Occasionally, improvement became most clinically apparent after the completion of the treatment. These horses were difficult to evaluate for their response to diclazuril, as they may have responded to supportive care, anti-inflammatory treatment or to time alone.

The adverse reactions reported in this investigation were few and could not be specifically linked to the compound. Increases in serum liver enzyme values could have been chance observations. The occurrence of colic in a horse may have been due to chance, may have been associated with the quantity of Clinacox powder administered at one time, or associated with the soy protein 'vehicle' of this formulation. Worsening of clinical signs with treatment ('treatment crisis') appeared to be more likely to occur in horses that exhibited severe neurological impairment. It remains possible that all adverse effects seen in this investigation are a direct result of the oral administration of diclazuril to these horses.

Limited work in other species has indicated minimal toxic effects at high doses for several consecutive days. Acute toxicity trials on rats, mice, dogs and hens failed to provide drug-induced mortality (Anon 1995). No clinical symptoms were apparent in the hen at doses up to 5000 mg/kg bwt. No mutagenic, teratogenic or carcinogenic effects have been demonstrated (Anon 1995). The findings of this clinical trial in the horse support the suspicion of low toxicity in this species.

Severe, acute hepatitis has been a concern articulated by some veterinarians who have administered diclazuril. To our knowledge, there are no clinical or *postmortem* reports that substantiate this concern. To our knowledge, no other clinical trial of diclazuril has been published. Although acute hepatic insult is a possibility, we have seen no direct evidence of liver damage. No toxic effects have been seen in dogs after repeated oral dosing of 5 and 20 mg/kg bwt over a 3 month period (Anon 1995). However, doses of 80 mg/kg bwt resulted in minor liver changes in dogs. These effects were reversible and disappeared after discontinuation of the medication (Anon 1995).

Tissue culture studies have shown diclazuril to be effective in killing related organisms (Lindsay and Blagburn 1994). Recently, such efficacy has been demonstrated against *Sarcocystis neurona* (Lindsay *et al*. 2000). At this time, it is unknown how diclazuril compares to treatment with standard therapy (pyramethamine and sulphadiazine). Synergy of diclazuril and pyrimethamine has been demonstrated in the treatment of mice with *Toxoplasma gondii*-induced encephalitis (Lindsay *et al*. 1995). Such an approach could be useful in the treatment of EPM.

Study limitations

Based on our investigational protocol, we believe that most of the horses treated with this compound were likely to have been truly affected with EPM. However, because definitive *antemortem* diagnosis of EPM remains difficult at this time, some of the animals treated may have had other conditions that either did not respond to this treatment, or that improved in a manner unrelated to the administration of diclazuril. A matched control group was not utilised in this study. Therefore, although there was a significant difference in the pre- and post-treatment neurological grades, this investigation does not account for improvement that could occur with the passage of time. There is also no group in this study that can be used for direct and objective comparison of this treatment to other forms of treatment or to an untreated group. Not all of the horses in this investigation had been previously diagnosed and treated for this disease. Horses in the present investigation that had been previously treated were diagnosed without the same criteria, may have been evaluated by clinicians other than those that had submitted the case for the present study, and were treated for varying periods. Interclinician differences in the neurological examination and interpretation process are another area of variability. An important follow-up investigation would include a comparison of standard treatment, diclazuril and other new treatments through which more specific conclusions might be made.

Conclusions

Based on the data accumulated in this investigation, we believe there is good evidence of positive clinical response to diclazuril in a group of animals that may have otherwise died or required euthanasia. Further studies are required to outline the optimal dosage, route of administration, adverse effects of treatment and clinical efficacy in comparison to other EPM therapies. Based on pharmacokinetic data, shorter oral treatment intervals (less than 8 days) would not be expected to provide adequate CSF levels to kill *Sarcocystis neurona* or related organisms. Combined treatment (pyrimethamine and diclazuril) could be effective in treatment of EPM, but needs to be rigorously evaluated. Direct comparison of efficacy and relapse rates to other therapies cannot be addressed until controlled investigations of these treatments are conducted. Based on these results, diclazuril has considerable potential as a primary treatment of selected horses with EPM that have been either refractory to or have relapsed after standard treatment.

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