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The effect of diminazene aceturate on cholinesterase activity in dogs with canine babesiosis

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ABSTRACT

A clinical trial was designed to evaluate the effects of diminazene aceturate and its stabiliser antipyrine on serum pseudocholinesterase (PChE) and red blood cell acetylcholinesterase (RBC AChE) in dogs with babesiosis. The trial was conducted on naturally occurring, uncomplicated cases of babesiosis ($n = 20$) that were randomly allocated to groups receiving a standard therapeutic dose of diminazene aceturate with antipyrine stabiliser ($n = 10$) or antipyrine alone ($n = 10$). Blood was drawn immediately before and every 15 minutes for 1 hour after treatment. Plasma PChE showed a 4 % decrease between 0 and 60 min within the treatment group ($p < 0.05$). No statistically significant differences were found between the treatment and control groups at any of the time intervals for PChE. There was an increase in RBC AChE activity at 15 min in the treatment group ($p < 0.05$). No significant differences were found between the treatment and control groups at any time interval for RBC AChE. In view of the difference in PChE, samples from additional, new cases ($n = 10$) of canine babesiosis were collected to identify the affect of the drug over 12 hours. No significant depression was identified over this time interval. The results suggests that the underlying mechanism in producing side-effects, when they do occur, is unlikely to be through cholinesterase depression.

Key words: acetylcholinesterase, *Babesia canis,* babesiosis, canine, diminazene aceturate, pseudocholinesterase.

INTRODUCTION

Babesia canis is a common intracellular erythrocyte protozoan parasite of dogs in South Africa[26], transmitted by ticks (*Haemophysalis leachi*). Babesiosis in dogs can be classified clinically into uncomplicated or complicated cases[11]. The drugs most commonly employed at the outpatients clinic of the Onderstepoort Veterinary Academic Hospital (OVAH) in the treatment of *Babesia canis* infections in dogs are trypan blue (Kyron Laboratories) and diminazene aceturate (Berenil®, Hoechst Ag-Vet). Trypan blue is an azonaphthalene dye in an aqueous solution (10 mg/mℓ) and is administered intravenously at a rate of 1 mℓ/kg (10 mg/kg). Berenil® is an aromatic diamidine and is available as 44.5:55.5 m/m diminazene and antipyrine (stabiliser) formulation given as a single intramuscular injection of 1 mℓ/10 kg (4.2 mg/kg diminazene, 5.24 mg/kg antipyrine)[24].

The choice of drug is determined by the severity of the case. Severe cases are usually treated with trypan blue because it is believed to have fewer side-effects, and less severe cases are usually treated with diminazene[16]. Side-effects reported following diminazene treatment in dogs include vomition[4,14,16], nausea[4,14,16,19], urination[1], nervous signs[4,8,16,25] and anaphylactic shock[4,16]. Some of the side-effects seen with the diamidines are thought to be due to over-stimulation of the parasympathetic nervous system[7,13,16,19,27]. A more recent report indicates that pentamidine, a diamidine, effectively inhibits cholinesterase (ChE) *in vitro*[2,23]. Conversely, El-Sawi and Ali[6] and Arowolo and Eyre[3] reported that diminazene aceturate had no effect on cholinesterase *in vitro*. However, in both of these studies actual levels of pseudocholinesterase (PChE EC 3.1.1.8: Enzyme Commission name and number) or acetylcholinesterase (AChE EC 3.1.1.7) were not measured[3,6]. Other babecides used in the treatment of canine babesiosis, such as imidocarb dipropionate[21,22] and phenamidine (T W Naudé, Onderstepoort Veterinary Institute, South Africa, pers. comm.) do inhibit ChE.

Serum PChE recovers more rapidly than red blood cell (RBC) AChE due to its production by the liver. Liver disease can affect recovery of PChE[15]. Because RBC AChE is an integral part of the red cell membrane, recovery takes longer. This, in effect, means that younger RBCs have higher concentrations of AChE[15]. Biochemical assays for RBC AChE and serum PChE are sensitive tests in identifying exposure/poisoning due to inhibitory substances[9,20]. The sensitivity of the test is improved by monitoring baseline values before exposure[10]. Inhibition of ChE by 20 % or more from the baseline is regarded as diagnostic for exposure/acute toxicity due to organophosphate compounds[10].

It appears that no direct measurement of blood levels of ChE has been carried out following diminazene administration[3,6]. If diminazene has a marked influence on cholinesterase, this could be serious for body homeostasis, especially in an ill animal[18]. The aim of the present clinical trial was to evaluate the effects of diminazene aceturate and its stabiliser antipyrine (Berenil®) on serum PChE and RBC AChE.

MATERIALS AND METHODS

Twenty naturally occurring, uncomplicated[11], confirmed (a blood smear) cases of canine babesiosis that met specific criteria, such as owner's consent, older than 6 months, body mass greater than 5 kg, haematocrit between 0.15 and 0.35 and no history of recent exposure or regular use of organophosphate or carbamate dips for tick or flea control, were admitted to the trial. These cases were randomly allocated to groups receiving a standard therapeutic dose of diminazene with antipyrine stabiliser (Berenil®, Hoechst Ag-Vet) (Group 1, $n = 10$) or antipyrine[c] (Phenazone powder, Lennon Limited) alone

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[c]2.36 g powder dissolved in 25 mℓ water and filtered through a 0.22 µm micropore filter into brown glass vial under sterile conditions.

Table 1: **Means and standard deviations for PChE and RBC AChE for Groups 1–3.**

	Group 1 (treatment) PChE (IU/ℓ)					Group 2 (control) PChE (IU/ℓ)				
Time interval (min)	0	15	30	45	60	0	15	30	45	60
Mean	3070	3032	2966	2999	2935[a]	4026	4010	3920	4003	3978
Standard deviation	1139	1142	1039	1094	1013	1527	1596	1686	1610	1665

	Group 1 (treatment) RBC AChE at 5.4 min U/g haemoglobin					Group 2 (control) RBC AChE at 5.4 min U/g haemoglobin				
Mean	71.3	85.2[b]	79.2	80.1	75.1	83.9	88.8	86.8	84.5	89.9
Standard deviation	11.9	17.4	10.5	11.5	13.2	20.5	22.9	18.4	19.7	21.9

	Group 3 (additional cases) PChE (IU/ℓ)			Group 3 (additional) RBC AChE at 5.4 U/g haemoglobin		
Time interval (min)	0	60	720	0	60	720
Mean	3225	3420	3787	75.7	82.2	96.7
Standard deviation	1567	1700	2131	18.2	23.4	38.7

[a,b]Statistically significant difference when compared to 0 min.

(Group 2, $n = 10$). Blood was drawn into evacuated potassium EDTA tubes (Becton Dickinson, Vacutainer Systems, Europe) at time 0 (baseline), 15, 30, 45 and 60 min respectively. All control cases (that received antipyrine alone) were treated with Berenil® following completion of the blood sampling.

The EDTA samples were centrifuged (Jouan B3.10 centrifuge, France) at 3000 rpm (1730 G RCF) for 5 min so as to separate red cells from plasma. The harvested plasma and red cell samples were stored (±30 days) at –20 °C for batch processing. PChE and AChE have been shown to be stable at –20 °C for 9 weeks[17]. On completion of the trial the samples were allowed to thaw and reach room temperature. A Boerhinger Mannheim kit (Cholinesterase, Kit no. MPR1 1447 297) for the Cobas Mira (S A Scientific Products, Randburg) using a modified automated method[12] was used to determine PChE levels.

Packed red cell samples were also allowed to thaw and reach room temperature. Red cell AChE determinations were conducted using a modified automated method[5] for the Cobas Mira. Controls for the methods were based on standard laboratory operating procedures.

Statistical evaluation of the data was performed using a commercial statistical package (Sigmastat, Jandel Scientific Software, USA). The data were tested for normality and equal variance by the Kolmogov-Smirnov and Levene's median tests, respectively. A one-way repeated measures analysis of variance was used to compare data in the same treatment group at different time intervals with pre-treatment values. The data between the 2 treatment groups were compared using one-way analysis of variance. *Post hoc* multiple comparisons were done using Bonferroni's *t*-test. Significance was accepted at $p < 0.05$. In view of the statistically significant difference in PChE in the treatment group between 0 min and 60 min, additional samples (Group 3, $n = 10$ new cases) were collected to identify possible depression over 12 hours. These additional data ($n = 10$) were collected from canine babesiosis cases admitted overnight to the outpatients section of OVAH. Samples were collected at 0, 60 and 720 min, and processed in the same manner as described above.

RESULTS

The arithmetic means and standard deviations of PChE, RBC AChE of Groups 1, 2 and 3 are presented in Table 1. A comparison between changes in repeated measurements of plasma PChE within the treatment group over time (0, 15, 30, 45 and 60 min) showed a statistically significant decrease between 0 and 60 min ($p < 0.05$). No statistically significant differences were found between the treatment and control groups at any of the time intervals for PChE. There was a statistically significant ($p < 0.05$) increase in RBC AChE activity at 15 min in the treatment group. No statistically significant differences were found between the treatment and control groups at any time interval for RBC AChE. A comparison between changes in repeated measurements of plasma PChE and RBC AChE within Group 3 (additional data) over time (0, 60, 720 min) revealed no statistically significant change.

DISCUSSION

The principal finding, treatment against control, indicated that diminazene does not depress ChE. The only statistically significant change occurred in Group 1 ($p < 0.05$) between the 0 min sample and 60 min sample. While this difference (4 %) may be statistically significant, one would have expected a marked decrease of at least 20 % in enzyme levels with acute poisoning/exposure over a 60 min period. However, the result may have been an indication of a more delayed depression of PChE over time, possibly due to a metabolite of diminazene. The data from the 2nd study (Group 3) indicated that there was no decreasing trend. This may to some extent support the work done by El-Sawi and Ali[6] and Arowolo and Eyre[3] that suggested, albeit indirectly, that diminazene aceturate has no cholinesterase-inhibiting ability.

The increase in RBC AChE levels rather than a decrease at 15 min may be explained by the finding that AChE levels in red blood cells are related to the age of red blood cells[28]. Splenic contraction and the release of reticulocytes into the circulation at the time of collection may explain this unexpected finding, but this could not be confirmed, as no reticulocyte counts were performed on the 15, 30, 45 or 60 min samples.

CONCLUSION

The results of this trial do not confirm the widely held view that Berenil® depresses ChE. The results do not prove that Berenil®, at the dose rate used in the treatment of canine babesiosis, does not cause side-effects, but suggests that when side-effects occur, the underlying mechanism is unlikely to be ChE depression.

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