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PHARMACOKINETICS OF HALOFUGINONE IN CATTLE

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SUMMARY

The pharmacokinetics of the antitheilerial drug halofuginone were evaluated in healthy calves following oral administration, at a dose of 1·2 mg/kg body weight, repeated after 48 h. The maximum plasma concentration after the first dose ranged from 3·8 to 7·7 ng/ml (6·5 ng/ml, mean) and occurred at between 12 and 32 h (22 h, mean). After the second dose, the maximum plasma concentration was 4·8–8·6 ng/ml (7·2 ng/ml, mean) occurring between 12 and 32 h (17 h, mean). The apparent terminal elimination half-life ranged from 24·2 to 28·9 h with a harmonic mean of 27·3 h. No significant difference was found in the apparent volume of distribution and the body clearance between the values calculated after the first dose and the two doses. The results show that the concentrations which persist in plasma from 8 to 120 h are above the in-vitro concentration of halofuginone required to reduce by 50% the proportion of lymphoblastoid cells containing *Theileria parva* schizonts (EC_{50}=3 ng/ml), but the maximum plasma concentrations are only about 46% of the in-vitro optimal effective concentration, EC_{80} (15 ng/ml).

INTRODUCTION

Although the intensive search for specific drugs against bovine theileriosis started about 40 years ago, it is only within the last 14 years that a breakthrough was made with the discovery of menoctone (McHardy, Haigh & Dolan, 1976; McHardy, 1978). Three compounds have now been developed and are currently available for the treatment of infections in cattle caused by *Theileria parva parva* (East Coast fever, ECF, which occurs in Africa) and *T. annulata* (Mediterranean or tropical theileriosis; which occurs in Africa, Asia and Southern Europe). These are the quinoxalinone compound, halofuginone (Njau & Mkonyi, 1981; Morgan & McHardy, 1982; Njau *et al.*, 1985; Kiltz & Humke, 1986; Mbwambo *et al.*, 1986; Chema *et al.*, 1987), originally developed as an anticoccidial drug (Ryley & Wilson, 1975), and two hydroxynaphthoquinone derivatives, parvaquone and buparvaquone (Morgan & McHardy, 1982; McHardy *et al.*, 1983, 1985; Dolan *et al.*, 1984; Mbwambo, Mkonyi & Chua, 1987; Dhar *et al.*, 1988).

From the published reports that have emerged to date, the effectiveness of these three drugs (judged by recovery rate, recrudescence of infections and therapeutic indices) seems to decrease in the order: buparvaquone, parvaquone and halofuginone.

This trend may be partly attributed to their intrinsic activities (Morgan & McHardy, 1982; McHardy & Wekesa, 1985) and to their pharmacokinetic characteristics. While recent studies have shown that buparvaquone in cattle has better pharmacokinetic properties than parvaquone (Kinabo & Bogan 1988) no such data on halofuginone have been published. The study of halofuginone in cattle by Humke (1985) was limited since the plasma concentration–time profile and the kinetic parameters were not reported.

The purpose of the present study was to evaluate the disposition kinetics of halofuginone in cattle after oral administration at the therapeutic dose given twice with a 48 h interdosing interval to reflect closely the typical regimen recommended for field use.

MATERIALS AND METHODS

Cattle

Six clinically healthy *Bos taurus* male calves weighing 118–205 kg were used. They were given a diet of hay and had free access to water.

Drugs

The drug formulation used was halofuginone lactate, Terit^{R} (a solution of 25 mg active substance/ml) donated by Hoechst AG, West Germany. Halofuginone for preparation of standard solutions (reference substance Batch 4L2074) was obtained from Hoechst AG, England.

Drug administration and blood sampling

The drug was administered at a dose of 1·2 mg/kg body weight as a single dose in two calves (pilot study), and as a double dose at an interval of 48 h in four calves. The calculated dose for each animal, which averaged 6·12 ml of the drug preparation, was diluted in 0·5 l of water and administered orally using a long-necked bottle. Blood samples were drawn from the jugular vein over a period of 0–24 h for the two calves which received a single dose, and over a period of 0–168 h for the calves which received two doses 48 h apart. The blood was centrifuged and plasma was collected and kept at −20°C until analysed.

Drug analysis

Concentrations of halofuginone in plasma samples were determined by high performance liquid chromatography after solid phase extraction (Kinabo, McKellar & Murray, 1989).

Pharmacokinetic analysis

The maximum plasma concentrations after the first dose (C_{max1}) and the second dose (C_{max2}) and their respective times of occurrence t_{max1} and t_{max2} were obtained by visual inspection of the data. The apparent terminal elimination rate constant (β) was calculated by logarithmic-linear regression of the terminal concentration–time data after the second dose. The apparent terminal elimination half-life ($t_{1/2\beta}$) was calculated as ln2/β. The total area under the plasma concentration versus time curve (AUC) was estimated by the trapezoidal rule, and extrapolated to infinity using the last measured concentration and β. The apparent volume of distribution during the terminal elimination phase (V_d area/F, where F is the fraction that reaches systemic circulation) was estimated

by the equation Dose/$\beta \cdot$ AUC, and body clearance (Cl_b/F) was calculated as Dose/AUC. In this study, we could not measure F (AUC oral/AUC intravenous) because the drug formulation was not suitable for intravenous administration. For all the kinetic parameters, values from each of the four animals that were given two doses of the drug were obtained and then averaged to provide the mean values. Data from the two calves which received a single dose were not used for calculation of the kinetic parameters because blood sampling was stopped 24 h after drug administration, at a time when the concentrations were within the C_{max1} range.

Statistical analysis

Differences between mean values were evaluated by the Student's t-test for paired data. A $P<0{\cdot}05$ was considered significant. The results are presented as mean ± SEM except for $t_{1/2\beta}$ which is given as the harmonic mean.

RESULTS

The plasma concentration versus time profile for halofuginone in the six calves is presented in Fig. 1. The drug was detectable in plasma (>1 ng/ml) 2 h after administration and persisted at detectable concentrations for up to 144 h. The C_{max1} was $6{\cdot}50 \pm 0{\cdot}92$ ng/ml and this was slowly attained after $22 \pm 5{\cdot}77$ h (t_{max1}, Table I). The C_{max2} averaged $7{\cdot}20 \pm 0{\cdot}87$ ng/ml with a t_{max2} of 17 ± 5 h (Table I), and these values did not significantly differ from the corresponding values obtained after the first dose. The apparent terminal elimination half-life ranged from 24·23 to 28·94 h, with a harmonic mean of 27·25 h (Table I). The mean total AUC calculated after the two doses was 1·86 times higher than that found after the first dose (Table I). The apparent volume of distribution and body clearance shown in Table I contain a measure of availability F, because the intravenous route was not used. For both parameters, no significant differences were found between the values calculated after the first dose and the two doses.

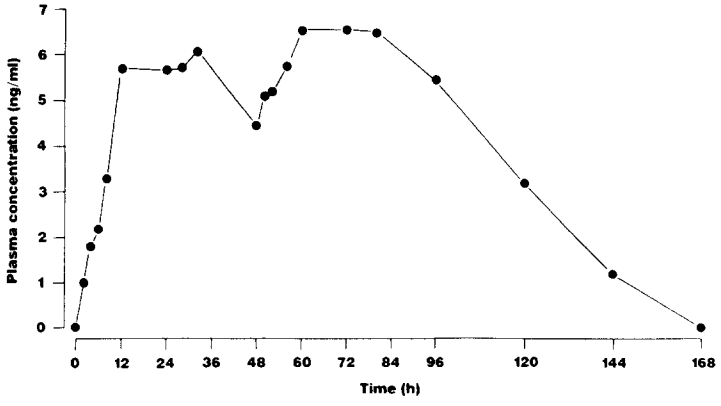

Fig. 1. Plasma concentration–time profile of halofuginone following oral administration at a dose of 1·2 mg/kg body weight, as a single dose in two calves, and as a double dose at an interval of 48 h in four calves. Each point from 0 to 24 h represents the mean of six animals and from 32 to 168 h represents the mean of four animals.

Table I

Disposition kinetics of halofuginone in calves following oral administration of the drug at a dose of 1·2 mg/kg body weight repeated after 48 h

Parameter	*Units*	*Calf number*				*Mean* ± *SEM*	
		30	*86*	*47*	*82*		
C_{max1}	ng/ml	3·8	7·7	6·8	7·7	6·50	±0·92
t_{max1}	h	12	12	32	32	22	±5·77
C_{max2}	ng/ml	4·8	7·0	8·6	8·3	7·2	±0·87
t_{max2}	h	12	12	32	12	17	±5·00
β (2)	h^{-1}	0·0241	0·0240	0·0286	0·0251	0·0254	±0·0011
$t_{1/2\beta}$(2)	h	28·77	28·94	24·23	27·62	27·25*	
AUC(1)	ng · h/ml	249	419	419	489	394	±51
AUC(2)	ng · h/ml	452	843	822	820	734	±94
V_d area/F (1)	l/kg	200	119	100	98	129	±24
V_d area/F (2)	l/kg	220	119	102	117	140	±27
Cl_b/F(1)	ml/min/kg	80·3	47·7	47·7	40·9	54·2	±8·9
Cl_b/F(2)	ml/min/kg	88·5	47·4	48·7	48·8	58·4	±10·0

C_{max1}, maximum plasma concentration after first dose; t_{max1}, time of C_{max1}; C_{max2}, maximum plasma concentration after second dose; t_{max2}, time of C_{max2}; β, hybrid rate constant for terminal phase of elimination; $t_{1/2\beta}$, terminal elimination half-life; AUC, area under the plasma concentration–time curve; V_d area/F, volume of distribution after extravascular administration; Cl_b/F, body clearance after extravascular administration; (1) calculated after first dose; (2) calculated after two doses; * harmonic mean.

DISCUSSION

These results suggest low bioavailability of halofuginone after oral administration to cattle. However, this does not necessarily mean poor absorption because it can be caused by metabolism of the drug in the gut or by rapid uptake and first-pass metabolism in the liver. Moreover, due to the ionic nature of halofuginone, its absorption from the gastrointestinal tract would not be expected to be very high. The prolonged time to maximum plasma concentration could be attributed to binding of the drug to gastrointestinal solid contents, from where it is slowly released for absorption. It could also be due to slow release from binding to high affinity sites in the liver, since the drug will traverse the hepatic portal system and the liver before reaching the systemic circulation.

The large apparent volume of distribution (V_d area/F > 1 l/kg) indicates that the concentrations of the drug in tissues are higher than in plasma. Note that this will hold true even if F was only 1%. It is thus probable that the low concentrations measured in plasma do not accurately mirror the amount of drug in the body. In cows given two doses of halofuginone (1 mg/kg body weight) at an interval of 48 h, 5 days after the first dose, the drug was not detectable in plasma but, in tissues such as the liver and kidney, the concentrations were high, ranging from 62 to 283 and 313 to 333 ng/g, respectively (Humke, 1985). These results strengthen the view that poor absorption cannot be confirmed on the basis of the plasma concentrations alone.

The high body clearance determined here is consistent with the large apparent volume of distribution. The lack of significant difference in the apparent volume of distribution

and the body clearance between the values calculated after the first dose and the two doses show that the distribution and elimination of halofuginone obeyed first-order kinetics.

The apparent terminal elimination half-life for halofuginone determined here may be described as long and is similar to that for buparvaquone (26·44 h) but much longer than that for parvaquone (11·12 h; Kinabo & Bogan, 1988). Nevertheless, because halofuginone seems to occur in higher concentrations in tissues than in plasma, it is evident that its terminal elimination half-life may be a poor indicator of the rate of its elimination at the cellular and organ level.

What we have found here is that the recommended two doses of 1·2 mg/kg body weight given 48 h apart are sufficient to maintain plasma concentrations that are above the in-vitro EC_{50} against *T. parva* (3 ng/ml; the concentration of drug required to reduce by 50% the proportion of lymphoblastoid cells containing schizonts; Morgan & McHardy, 1982) from 8 to 120 h. These plasma concentrations seem to be of good therapeutic significance since, after administration of the drug to cattle suffering from ECF, the pattern of clinical response (Schein & Voigt, 1979, 1981; Njau *et al.*, 1985; Kiltz & Humke, 1986) appears to parallel the concentration–time profile seen here.

Nevertheless, these concentrations may not be optimal for clinical response in view of the fact that the maximum plasma concentrations are only about 46% of the in-vitro optimal effective concentration (EC_{80}=15 ng/ml; Morgan & McHardy, 1982). This may account for the high incidence of recrudescence of infection that has been observed (Morgan & McHardy, 1982; Dolan, 1986). It therefore seems probable that increasing the dose to 2 mg/kg body weight (Schein & Voigt, 1981; Humke, 1985) or increasing the dosing frequency (Njau *et al.*, 1985) could improve the response. These strategies would probably prove successful only if the concentrations in the lymphoid tissues and general circulation (lymphocytes) are optimal. These are the predilection sites for the schizont, the most pathogenic stage of the parasite (Mehlhorn & Schein, 1984) and the one which appears to be most affected following treatment with halofuginone (Schein & Voigt, 1979; Mehlhorn *et al.*, 1981; Njau *et al.*, 1985; Dolan, 1986). The mode of action of the drug is unclear, but it is considered that it acts primarily by destroying parasitized lymphocytes and consequently the schizonts, which are bound by a single membrane only, are destroyed upon release to the extracellular environment (Mehlhorn *et al.*, 1981).

Because involvement of the gastrointestinal and respiratory systems is a common feature in ECF, it is probable that infected animals will exhibit different kinetics from healthy animals and large variations between animals depending on the severity of the disease may be seen. This may explain why recovery rates in infected animals are lower when the drug is administered at late stages than at early stages of the disease (Morgan & McHardy, 1982; Njau *et al.*, 1985; Chema *et al.*, 1987). Further studies using animals suffering from ECF would provide a basis for optimal therapy since dosage regimens may need to be modified in proportion to the seriousness of the disease in a given animal.

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