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Chemotherapy of East Coast fever

Treatment of infections induced by isolates of *Theileria parva* with halofuginone

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Summary

Cattle were infected with three isolates of *Theileria parva* and treated with halofuginone lactate during acute clinical disease. The health, weight gain and carrier state of the cattle were monitored for 15 months. Limited treatment rapidly reduced fever and parasitosis but parasite recrudescences occurred and 12 out of 21 treated cattle died. Persistent carrier states were identified with two *T. p. parva* isolate infections and a transient carrier state with *T. p. lawrencei*. Three cattle which died from a chronic wasting syndrome during the follow-up period showed exhaustion of lymph nodes but no *Theileria* macroschizonts were detected in any tissue.

Key words: *Theileria parva;* chemotherapy; halofuginone; carrier state; weight changes.

Introduction

The antitheilerial activity of the quinazolinone anticoccidial, halofuginone hydrobromide (Stenorol, Roussel Uclaf/Hoechst AG), was first reported by Schein and Voigt (1979) and confirmed by Uilenberg et al. (1980). Until very recently no treatment had been available for East African theileriosis, caused by *Theileria parva parva* (East Coast fever, ECF) and *T. p. lawrencei* (Corridor disease). The discovery of the antitheilerial activity of hydroxynaphthoquinone, menoctone (McHardy et al., 1976) and later of its analogue parvaquone (McHardy et al., 1980) showed that clinical ECF could be treated. The history of the search for effective antitheilerial drugs has been reviewed (Wilde, 1967; Dolan, 1981). During the developmental studies on parvaquone (Clexon, Wellcome) an in vivo screening procedure was proposed, using three *T. p. parva* and

two *T. p. lawrencei* isolates as stabilates (Dolan et al., 1984a), which should have a predictive value for field use of candidate compounds. The weight changes, parasite carrier states and disease manifestations of the parvaquone treated and recovered cattle were monitored for 19 months and the observations have been reported separately (Dolan, 1986). Parvaquone is the only drug available for the treatment of theileriosis, therefore new compounds will have to be compared with it. The recommended treatment with this drug is two intramuscular injections of 10 mg/kg 48 h apart, although further treatments may be administered if necessary. In this examination of halofuginone an experimental design, similar to that used in the assessment of parvaquone, was employed using two treatments in an attempt to control the diseases induced by three of the five proposed isolates. The subsequent health, weight gains and carrier state of the cattle was monitored for 15 months.

Materials and Methods

Cattle

Bos Taurus (Friesian) steers of 18–24 months were purchased from a farm in Kenya which practised good acaricide control and which had no recent history of theileriosis. The cattle contained no antibodies to *T. p. parva* schizont antigen in the indirect fluorescent antibody test (IFAT) (Burridge and Kimber, 1972). Before infection they were treated with oxyclosamide/levamisole (Nilzan, Coopers) and imidocarb dipropronate (Imizol, Wellcome). They were subsequently treated every three months with oxyclosamide/levamisole. The cattle were maintained on a 3/4 day dipping regime in dioxathion (Delnav DFF, Coopers) except during periods of tick feeding.

For two weeks prior to infection and for five weeks after infection they were held in a partially covered yard and fed on hay, a small quantity of concentrates and water ad libitum. Then they were moved to pasture but their diet was supplemented as above during the dry seasons.

Parasites

Theileria p. parva (Mbita) stabilate 169, isolated in Kenya, *T. p. parva* (Entebbe) stabilate 133, isolated in Uganda and *T. p. lawrencei* (Manyara) stabilate 140, isolated in Tanzania were inoculated in 1.0 ml volumes below and in front of the left ear. The details of isolation, history and disease characteristics of these three stabilates have been described elsewhere (Dolan et al., 1984a).

Tick pick-up attempts were made by the application of 2,000 clean *Rhipicephalus appendiculatus* nymphs from the colony at V.R.D. to the washed ears of two cattle from each of the infected cattle groups. When these ticks had moulted to adults and hardened, 500 pooled from the two cattle from each group were applied to the ears of susceptible cattle and the response monitored.

Haematology

Total leucocyte (WBC) counts, red blood cell (RBC) counts and packed cell volumes (PCV) were estimated twice each week prior to infection, every second day from day of infection (Day 0) to Day 35 and once a month to the end of the study. These values were obtained using an electronic particle counter (Coulter Electronics, Model ZB1).

Serology

The IFAT was used to screen for antitheilerial antibodies before infection, once each week to Day 35 after infection then monthly to the end of the study.

166

Lymph node biopsy

Lymph node biopsy smears were prepared daily from the left parotoid lymph node from Day 5 after infection. Once macroschizonts were detected the right prescapular lymph node and tail tip blood smears were prepared.

Lymph node biopsy smears were prepared daily until Day 35 then from any animal that showed clinical disease. Blood smears were prepared weekly for the duration of the study. All smears were fixed in methyl alcohol, stained with Giemsa's stain and examined.

Rectal temparature and weight

Rectal temperature was recorded daily. Weights were recorded prior to infection, weekly to Day 35, then monthly.

Post mortem examination

Full post mortem examinations were carried out as soon as possible after death. Impression smears were prepared from lymph nodes, spleen, heart, lung, adrenal and pituitary glands, eye and brain. Formalin fixed tissues were taken from these tissues of all cattle at post mortem. Any tissue which contained parasites or showed any abnormality on gross examination was processed, stained with haematoxylin and eosin and examined.

Experimental design

Thirty-two cattle were randomly allocated to four groups, three of nine cattle each and one of five. The groups of nine were infected, one with each of the three stabilates. On the third and fifth day of fever (39.5°C or higher) after the appearance of macroschizonts seven cattle in each stabilate group were treated orally with 1.2 mg/kg of halofuginone lactate (dl-trans-7-bromo-6-chloro-3-3 (3-hydroxy-2-piperidyl) acetonyl-4 (3H)-chinazolinon-lactate) (Terit, Hoechst AG) in one litre of water. The remaining two cattle in each group were untreated controls. The group containing five cattle was not infected but the animals were treated with halofuginone and acted as controls for all parameter measurements for the infected cattle and as susceptible sentinel cattle during the follow-up study while the cattle were at pasture.

At four, seven, ten and fifteen months after infection clean nymphal *R. appendiculatus* were applied to the washed ears of two cattle from each infected group (one animal from Group III at ten and fifteen months) and at ten months to the ears of two cattle from the uninfected group. These ticks were used in transmission attempts as described.

Results

The results of infection of cattle with three isolates of *T. parva*, and treatment with halofuginone lactate are summarized to Day 35 in Table 1. Three cattle survived from each of the three treated groups while all infected control cattle died. In Group I (*T. p. parva*, Mbita) one treated animal (number 45) died prior to the administration of the second treatment dose. In general there was a rapid response to treatment with marked degeneration of the intracellular schizonts in 24 to 48 h. Fever was reduced within 24 h of the second treatment. Of the cattle which recovered one had a transient parasitosis after ten days. This animal also had a leucopenia which began to recover from Day 20, three days after the second treatment. The two other recovering cattle had a leucocytosis following treatment. Of the four cattle which died, two had a partial reduction of macroschizonts for a few days. One animal had a low parasitosis for seven days after treatment then a resurgence which resulted in death on Day 29. The dying

Table 1. Disease and treatment responses of cattle infected with three isolates of *Theileria parva* and treated with halofuginone

Parasite isolate	Animal number	Time in days to:				
		appearance of macro-schizonts	appearance of fever	installation of treatment	recovery	death
Theileria	45	6	15	17	–	19
p. parva	50	10	14	16	–	29
(Mbita I)	57	10	14	16	19	–
	62	11	15	17	21	–
	65	11	14	16	–	24
	547	10	16	18	–	26
	592	9	13	15	7	–
	Mean±s.e.	9.43±0.69	14.43±0.37	16.43±0.37	22.33±2.40	24.50±2.10
Control	42	10	14	–	–	22
	573	8	15	–	–	23
Theileria	44	10	12	14	–	20
p. parva	49	10	13	15	–	24*
(Entebbe)	56	8	12	14	34	–
	64	7	13	15	–	23*
	66	10	14	16	–	25
	537	10	14	16	18	–
	632	10	14	16	19	–
	Mean±s.e.	9.29±0.47	13.14±0.34	15.14±0.34	25.67±5.17	23.00±1.08
Control	41	10	12	–	–	17
	611	9	11	–	–	19
Theileria	43	7	9	11	–	26
p. law-	47	5	9	11	–	26
rencei	52	7	10	12	29	–
(Manyara)	60	6	10	12	–	16
	63	7	10	12	–	19
	67	7	10	12	17	–
	597	8	10	12	27	–
	Mean±s.e.	6.71±0.36	9.43±0.30	11.71±0.18	24.33±3.71	19.45±2.36
Control	40	7	8	–	–	14
	597	7	10	–	–	15

* Killed in extremis

cattle all had leucocyte counts below 50% of pre-infection values before death although three had shown a transient leucocytosis following treatment. One animal (number 65) showed nervous signs of twitching and high stepping for 48 h prior to death.

In Group II (*T. p. parva,* Entebbe) one animal (number 537) died prior to the second treatment. The responses to treatment were generally similar to those in Group I except that fever was reduced in 24 h after the first treatment in six cases, although two had secondary fever episodes. Of the cattle which recovered, one (number 56) showed a low parasitosis (with secondary fever) for 17 days following the second treatment. The leucocyte count was depressed during most of this parasitosis only returning to pre-infection value 14 days after treatment. Another recovering animal (number 632) showed no macroschizonts from the day of second treatment but it had a persistent leucopenia of below 50% of its pre-infection value which began to recover six days after completion of treatment. The three cattle which died following two treatments all showed a reduction in parasitosis and a leucocytosis which were followed by a recrudescence of macroschizonts and leucopenia. Two became recumbent and were destroyed (Table 1).

In Group III (*T. p. lawrencei,* Manyara) macroschizonts, fever and death were recorded earlier than in the other groups (Table 1) and treatment was applied significantly earlier. In recovering cattle fever was reduced after 48 h in two cattle and after 72 h in the other five, although two had secondary fever episodes. Two of the dying cattle had their fever reduced in 24 h but it recurred in 24 and 48 h, the third had fever reduced in 72 h and the fourth showed no fever reduction. Macroschizonts were not detected from 72 h after completion of treatment in recovering animal (number 67) although its leucocyte count was slow to recover. The other two recovering cattle had low macroschizont parasitoses for seven and twelve days. The animals with parasites for 7 days showed a reactive leucocytosis while the other animal had a leucopenia which persisted while macroschizonts were detectable. Of the dying cattle, two died without appreciable effect upon macroschizonts (numbers 60 and 47), one with a reactive leucocytosis. Another (number 43) had no detectable macroschizonts for 5 days following first treatment. The leucocyte count was recovering, then macroschizonts reappeared, their numbers increased rapidly, the leucocyte count fell and the animal died 15 days after initiation of treatment. The fourth dying animal (number 63) had a marked reduction in parasitosis and a leucocytosis following treatment then a fatal recrudescence of macroschizonts with leucopenia.

Post mortem examinations of both treated and untreated cattle which died up to Day 35 all showed lesions typical of theileriosis. One animal (number 43) in Group III also had a secondary bacterial pneumonia. Of the nine cattle which survived to Day 35 following treatment, one animal died in Group II (number 56) and two in Group III (numbers 67 and 52) (Table 2). These three cattle all showed progressive weight loss, became recumbent, although alert and died (one was destroyed) in an extremely emaciated state. No macroschizonts were detected on post mortem smear examination. However, lymph nodes from all three animals showed evidence of exhaustion with a reduction in cellularity, loss

Table 2. Summary of cattle numbers, transmission attempts and deaths from Day 35 to 450

Parasite isolate	Cattle no.	Transmission at:				Day of death
		4	7	10	15	
T. p. parva (Mbita)	3	+	+	+	+	48
T. p. parva (Entebbe)	3	+	+	+	+	149/174
T. p. lawrencei (Manyara)	3	–	–	+	–	0
Control	5	N/D	N/D	–	N/D	0

+ = Successful transmission of theileriosis
– = No transmission
N/D = Not done

of germinal centres and few cells in mitosis. Animal 56 from Group II and animal 67 from Group III showed infiltrative lesions in the kidney cortex appearing on the surface as pale creamy lesions sometimes depressed, 2–3 mm in diameter, extending usually in a wedge shape to the cortico-medullary junction and consisting of mononuclear (lymphocytic) cells.

Transmission attempts from the recovered cattle were successful from the Groups I and II cattle at all attempts but only at 7 months from Group III (only one animal available from Day 174) (Table 2). No antibodies were detected in the Group I cattle from 12 months while the Group II survivors had titres of 1:40 and 1:2560 (1:160 is positive) at 12 months and the latter animal was positive at 15 months. The Group III survivor had a titre of 1:640 at 12 and 15 months. No antibodies were detected in the control cattle and no disease was transmitted by clean ticks fed at 10 months. No piroplasms were observed in blood smears from these cattle from 2 months after infection.

All cattle groups, including the control cattle, lost weight during the first 2 months of the experiment. The Group II and III survivors recovered most rapidly and at 12 months showed a 30% (one animal) and 20% gain, respectively. Both the control and Group I cattle had gained 15% of their starting weight.

Discussion

Of 21 cattle infected with isolates of *T. parva* and treated with halofuginone, two died before the administration of the second treatment and another two died without any appreciable effect upon the course of the disease. Marked effects were observed on the parasitosis and the disease patterns of the remaining 17 cattle, although only 9 survived beyond Day 35. The experiment was designed as a severe test of compound activity using virulent isolates and a limited treatment regime. It is likely that more cattle would have survived if further treatments had been administered but the experiment was to compare halofuginone with an earlier study of parvaquone in the treatment of selected

isolates using a two dose regime. In general the effects of the treatment were very similar to those reported from earlier studies with halofuginone (Schein and Voigt, 1979; Uilenberg et al., 1980; Morgan and McHardy, 1982) but survival with these more virulent isolates was poorer. Halofuginone lactate (in starch tablets) was used in this experiment and by Schein and Voigt (1981) to treat *T. p. parva* (Muguga) infection, all other studies have been conducted using the hydrobromide salt. The lactate preparation is water soluble and better tolerated by cattle (Schein and Voigt, 1981).

Comparing halofuginone with parvaquone in the treatment of the diseases induced by these three isolates, there were differences in the character of the disease induced by the stabilates, in the responses to treatment and in survival (Dolan et al., 1984a). In an attempt to standardize the stage and clinical severity of disease at which treatment was applied, the third day of fever after the appearance of macroschizonts was chosen for comparative studies. This resulted in later treatment with halofuginone for both *T. p. parva* isolates, 16.43±0.37 days compared with 12.86±0.34 and 15.14±0.34 compared with 12.52±2.50, respectively, which was significantly later for *T. p. parva* (Mbita I). In the parvaquone experiment the cattle were of the same age and weight range but from a different source and 25% were of the Ayrshire breed. Thus animal effects may have contributed to the observed differences. It is unlikely that the stabilates varied in storage in the time between experiments nor is it thought that the infective dose varies when using the same volume of well mixed equilibrated stabilate (Dolan et al., 1984b).

The changes in total leucocyte counts were not as consistent as in previous chemotherapy studies using halofuginone or parvaquone where treatment generally resulted in a reactive lymphocytosis (Morgan and McHardy, 1982; Dolan et al., 1984a). However, a continuing depression of the leucocyte count was generally associated with poor prognosis. The more idiosyncratic response of cattle to treatment with halofuginone in this study might reflect also variation in drug absorption and distribution. Despite the later treatment of the *T. p. parva* (Mbita I) isolate, survival to Day 35 and long-term survival were identical with both halofuginone and parvaquone treatments (Dolan et al., 1984a; Dolan 1986). But parvaquone was superior to Day 35 in treating the other two isolates. Four halofuginone treated cattle died in each of the *T. p. parva* (Entebbe) and *T. p. lawrencei* (Manyara) isolate groups compared with one in each group following paravaquone treatment. Although complicating factors such as viral respiratory infections, abscess formation (controlled by sterile biopsy technique) and *Babesia* parasitaemias (controlled by pre-treatment with imidocarb dipropronate) were not found in the halofuginone treated cattle.

No *Theileria* macroschizonts were observed in impression smears or histological sections examined from the three halofuginone treated cattle which died after Day 35. Nonetheless, the lymphocytic infiltrations in the kidneys of the animals from Groups II and III and the exhausted appearance of the lymphoid

organ resembled the lesions seen in cattle which died during the follow-up period of parvaquone treated animals (Dolan, 1986). The gradual decline of these cattle was also reminiscent of that seen in the paravaquone study. Transmission of infection through clean ticks also followed the earlier pattern, with fatal disease being transmitted from the *T. p. parva* infected cattle and a transient carrier state being detected with *T. p. lawrencei* (Manyara). Thus, neither halofuginone nor parvaquone sterilized the infections, and the case for the occurrence of a carrier state following recovery from *T. p. parva* infection (Bevan, 1924; Dolan 1986) receives further support from these results.

The experiment was also a test of the *T. p. parva* isolates as a screening panel for the evaluation of potential antitheilerial compounds. The original panel consisted of five isolates from which the *T. p. parva* (Pugu II) isolate, which was most responsive to parvaquone treatment and did not produce a carrier state, and the *T. p. lawrencei* (Mara II) isolate, which was generally similar to *T. p. lawrencei* (Manyara) were not used. The isolates, apart from showing some variation in development of disease, possibly due to cattle effects, behaved reliably and reproduced the carrier state patterns of the earlier study (Dolan, 1986). Their virulence, geographical origin and their buffalo and cattle sources all recommend them as a valuable screen for candidate compounds.

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