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COMPARATIVE EFFICACY OF DIMINAZENE DIACETURATE AND IMIDOCARB DIPROPIONATE AGAINST *BABESIA EQUI* INFECTION IN DONKEYS

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ABSTRACT

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Diminazene diaceturate (Berenil, Hoechst) at 12 mg/kg intramuscularly (i/m) and repeated after 24 hours controlled the rising parasitaemia of *Babesia equi* infection in four out of five splenectomised donkeys. The drug was more effective in the early stages of the disease and had a prophylactic effect for at least 30—35 days.

A new babesicide, imidocarb (Imizol, Burroughs Wellcome), was 100% effective in three splenectomised donkeys at 5 mg/kg, i/m and repeated after 48 hours. However, imidocarb at 5 or 2 mg/kg i/m with a single injection was only partially effective or ineffective.

INTRODUCTION

The list of drugs against equine babesiosis includes trypan blue, Acaprin, Babesan, Pirevan, euflavin, phenamidine, quinine dihydrochloride, Berenil, oxytetracycline and others (Kirkham, 1969), but none are as effective against *B. equi* as they are against the large babesia *B. caballi*. Most of the drugs improve the clinical signs but do not completely eliminate the infection. Practical disease-control measures can usefully be undertaken with a drug that will completely eliminate the infection.

The present investigations deal with the therapeutic activities of diminazene diaceturate and imidocarb dipropionate against experimental *B. equi* infection in splenectomised donkeys.

MATERIALS AND METHODS

Animals

Sixteen donkeys, two to four years old, of nondescript indigenous breed and of both sexes were used. The animals were kept in tick-free stables.

Babesia equi strain

B. equi was isolated from an outbreak of babesiosis at the Equine Breeding Stud, Hissar (Gautam and Dwivedi, 1976) and was later maintained in indigenous donkeys by syringe passage of infected blood. These carrier donkeys were then splenectomised employing the technique of Dennig and Brocklesby (1965) to produce a clinical disease with high parasitaemia.

Haematological studies

The determinations of haemoglobin (Hb) and packed cell volume (PCV) were carried out following the methods of Schalm et al. (1975). The percent parasitaemia was recorded from examination of Giemsa-stained blood smears.

Experimental drugs

Berenil* and Imizol** were used. Berenil is the trade name for diminazene diaceturate (4,4'-diamidino-diazoaminobenzene-diaceturate), available as yellow granules readily soluble in water. Imizol is the trade name for imidocarb and is available as 12% w/v solution. Imidocarb, 3,3'-bis-(2-imidazolin-2-yl) carbanilide dipropionate, is one of the dibasic carbanilide compounds having a babesicidal action and is related structurally to aromatic diamidines.

Treatment schedule

Sixteen donkeys were divided into five groups. Group I had five donkeys, Group V had two donkeys and Groups II—IV had three donkeys each (Table I). Berenil at 12 mg/kg was administered intramuscularly (i/m) to animals of Group I and the dose was repeated after 24 h. Imizol at 2 mg/kg and 5 mg/kg i/m was administered to animals of Groups II and III, respectively. Group IV received Imizol at 5 mg/kg i/m and the dose was repeated after 48 h. The two animals in Group V served as untreated infected controls.

Seven days after the completion of the treatment, the treated donkeys were given 2 ml of cortisone (Decadron, Merck Sharp and Dohme) i/m daily for four days as an immunosuppressant and observed for recrudescence of

*Berenil, Hoechst Pharmaceuticals Ltd., Bombay, India.
**Imizol, Burroughs Wellcome and Co., Gt. Britain.

TABLE I

Efficacy of Berenil[1] and Imizol[2] against *Babesia equi* infection in splenectomised donkeys

Group	No. of animals	Drug	Dose (mg/kg, i/m)	No. of injections	Treatment interval (h)	No. dead	Effect of challenge
I	5	Berenil	12	2	24	1 died	Relapse in 2 and recovered
II	3	Imizol	2	1	—	All died	—
III	3	Imizol	5	1	—	1 died	Relapse in 1 and recovered
IV	3	Imizol	5	2	48	All survived	No relapse
V	2	Untreated infected controls	—	—	—	Both died	—

[1] Berenil, Hoechst Pharmaceuticals Ltd., Bombay, India.
[2] Imizol, Burroughs Wellcome and Co., Gt. Britain.

parasitaemia, if any. At this time, 100 ml of blood from each treated donkey was sub-inoculated i/m into clean susceptible splenectomised donkeys to see if treated animals had become free of infection or not. The animals of Groups I, III and IV were also challenged i/m with 100 ml blood showing 50% parasitaemia 30 days after completion of the treatment.

RESULTS

The results are summarised in Tables I—III. Among Berenil-treated donkeys of Group I, one died on the 29th day after infection showing 89% parasitaemia (Table II). No relapse of the disease occurred after cortisone injection and the sub-inoculation test was negative.

All Imizol-treated donkeys of Group II died. During treatment, the animals showed 10—32% parasitaemia which declined initially after treatment but rose again later and all animals died 9—23 days after treatment with 63—91% parasitaemia (Table III).

One Imizol-treated donkey in Group III died 38 days after treatment. The remaining two treated donkeys showed no relapse of the disease even after cortisone injection. The sub-inoculation test was also negative. The details are given in Table III.

All Imizol-treated donkeys in Group IV survived. No relapse occurred after cortisone administration and the sub-inoculation test was negative. The details are given in Table III. Imizol at 5 mg/kg in two doses caused some transient side-effects like dyspnoea, salivation, colicy pains and diarrhoea. These effects were not serious, persisted for one to two hours only and did not warrant any treatment.

TABLE II

Efficacy of Berenil[1] (12 mg/kg, i/m) in experimental *Babesia equi* infection in splenectomised donkeys

Animal No.	Treatment (days post infection)	Parasit. (%) when treatment started	Max. para-sit. (%)	Haemoglobin (gm%)		Packed cell volume (%)		Result	Effect of challenge
				Day of treatment	Min. after treatment	Day of treatment	Min. after treatment		
1	5 and 6	39	78	9.4	4.2	29	15	Survived	Relapse and recovered
2	5 and 6	41	89	9.8	4.0	29	13	Died 29th day after treatment	—
3	6 and 7	29	42	7.5	5.8	28	17	Survived	No relapse
4	4 and 5	16	37	9.8	4.4	29	14	Survived	Relapse and recovered
5	4 and 5	14	21	8.8	7.2	28	23	Survived	No relapse
6	Untreated infected controls	—	85	—	4.0	—	15	Died 6th day after infection	—

[1] Berenil, Hoechst Pharmaceuticals Ltd., Bombay, India.

TABLE III

Efficacy of Imizol[1] against experimental *Babesia equi* infection in splenectomised donkeys

Group No.	Animal No.	Treatment (days after infection)	Dose (mg/kg, i/m)	Parasit. (%) when treatment started	Max. parasit. (%)	Haemoglobin (gm%)		Packed cell volume (%)		Result	Effect of challenge
						Day of treatment	Min. after treatment	Day of treatment	Min. after treatment		
II	1	5	2	10	91	8.5	4.0	28	11	Died Day 23[2]	—
	2	7	2	30	63	5.2	4.2	16	15	Died Day 9	—
	3	8	2	32	83	9.0	4.0	29	13	Died Day 18	—
III	4	7	5	44	90	6.0	2.8	19	8	Died Day 38	Relapse in 1 and recovered
	5	6	5	37	37	9.8	4.8	30	16	Survived	
	6	7	5	16	34	7.0	5.1	22	16	Survived	
IV	7	8, 10	5,5	28	33	10.0	8.0	31	25	Survived	No relapse
	8	7, 9	5,5	42	49	7.8	6.4	27	24	Survived	
	9	6, 8	5,5	23	28	10.0	8.2	33	26	Survived	
	10	Untreated infected controls	—	—	87	—	4.8	—	18	Died Day 6	

[1] Imizol, Burroughs Wellcome and Co., London, Gt. Britain.
[2] After infection.

No recrudescence of parasitaemia following cortisone administration and blood sub-inoculation was observed in the treated animals of Groups I, III and IV. Also, the challenge infection following treatment of these animals caused mild relapses in about 50% of the animals but all of them recovered spontaneously afterwards.

DISCUSSION

Berenil proved quite effective, and the donkey which died in this group was given the drug when showing 41% parasitaemia. Probably, much damage had already been done and the animal died of anaemic anoxia.

Absence of relapse of the disease following cortisone administration and negative blood sub-inoculation results indicated the possible sterilizing effect of Berenil on *B. equi.*

The efficacy of Berenil was earlier reported by Joyner et al. (1963) in doses of 3—5 mg/kg against cattle babesiosis. Dennig (1965) reported that Berenil was the only drug that was successful against mild-to-moderate infection of *B. equi* in horses and donkeys, but that it was not effective against acute infection. The efficacy of Berenil against *B. caballi* or *B. equi* was further confirmed by many recent reports (Kirkham et al., 1966; Kirkham, 1969; Zavagli, 1969; Corbella, 1970; Gautam and Dwivedi, 1976).

It was of interest to note that after the first dose of Berenil, the parasitaemia continued to rise until, or even after, the second injection. This could suggest that some time was required for initiation of drug action on the parasites and their multiplication. The parasitaemia declined two to three days after treatment, and the animals recovered. In a pilot study conducted earlier by us, Berenil at a lower dosage was not effective and the present treatment schedule of two doses of 12 mg/kg 24 h apart was very effective.

Two-dose therapy with Imizol at 5 mg/kg was quite effective in bringing about not only clinical recovery but also complete elimination of the parasites. Absence of relapse following cortisone administration and negative blood sub-inoculation results further substantiated the efficacy of Imizol in eliminating the carrier status of the treated animals.

The present study confirms the findings of Kirkham (1969) and Carbrey et al. (1971) that Berenil and Imizol were effective against *B. equi.* However, a low dose schedule at 2 mg/kg of Imizol given on alternate days during a 40-day period, along with oral feeding of chlortetracycline daily for 28 days at 10 mg/kg, and at 16.5 mg/kg for another 12 days during the same 40 day period, was ineffective, as reported by Taylor (1973).

The time interval between doses seems to be important. Four i/m doses of imidocarb, 72 h apart at 4 mg/kg cleared *B. equi* from horses but not from the infected donkeys, which died even after treatment (Frerichs et al., 1973; Baturina and Lutsuk, 1975). Similarly, 4 i/m injections of imidocarb at 5 mg/kg, 72 h apart, also sterilised the *B. equi* infection in horses (Petrovskii, 1975).

Observations of these workers indicated that the time interval between the doses of imidocarb should be reduced while treating *B. equi*-infected donkeys. Hence, we chose two doses of 5 mg/kg administered 48 h apart and this worked very well.

The complete elimination of the infection was achieved by Imizol and Berenil in the animals of Groups I, III and IV, as indicated by the cortisone and blood sub-inoculation experiments. The mild relapses and the spontaneous recovery that were observed in these animals following challenge infection after the treatment could probably indicate the protective role of a sterile immunity in this disease.

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