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STUDIES ON IMIDOCARB DIHYDROCHLORIDE IN EXPERIMENTAL BABESIA BIGEMINA INFECTION IN CALVES (*)

By

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Summary

Four chemotherapeutic trials in Iran with the babesicide 3,3-bis- (2-imidazoline-2-yl) carbanilide dihydrochloride (Imidocarb dihydrochloride) are reported. Subcutaneous administration at a dose of I mg/kg proved highly effective in controlling experimental infection with a local virulent strain of *Babesia bigemina* in calves. While treated calves were apparently sterilized of the infection, they subsequently (100 days) were resistant to clinical re-infection despite the development-of a low parasitaemia.

No toxic effects attributable to the administration of the therapeutic dose of I mg/kg Imidocarb dihydrochloride were observed. Only mild and transient side effects were observed at 5 mg/kg and at 10 mg/kg; one of four calves died 12 days after the injection. The compound was considered to have a satisfactory therapeutic index and was shown to be less toxic and more effective in the treatment of *B. bigemina* infection than the therapeutic *N,N*- (dimethylquinolylium-methylsulphate-6)-urea (Acaprin), currently available in Iran.

INTRODUCTION

Following the report of Schmidt, Hirt & Fischer (1969), identifying the babesicidal activity of basically substituted carbanilides, Beveridge (1969) con-

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cluded that one member of this series of compounds, 3,3-bis- (2-imidazolin-2-yl) carbanilide dihydrochloride (Imidocarb dihydrochloride), was a highly effective babesicide against *B. rodhaini* in mice. Brown & Berger (1970), Callow McGregor (1970), and Mimioglu, Goksu & Ulutas (1972) have demonstrated the therapeutic activity of Imidocarb dihydrochloride against experiemental *B. bigemina* infection, and Callow & McGregor (1970) also demonstrated a chemoprophylactic activity. This was subsequently confirmed under field conditions by Roy-Smith (1971).

Babesia bigemina infection is prevalent in Iran and causes a high mortality. This paper details work with Imidocarb dihydrochloride in experimental infection of calves with a local virulent strain of *B. bigemina.* The aim was to investigate the therapeutic and chemoprophylactic activity and safety of the compound under Iranian conditions. Thee therapeutic activity of Imidocar dihydrochloride was also compared with that of *N,N*-(dimethylquinolylium-methylsulphate-6-)-urea (Acaprin), the currently available medicament in Iran.

MATERIALS AND METHODS

Experimental animals

Sixty-four calves (57 Friesian and 7 Sarabi), aged 4 to 6 months, were used in the experiments. They were purchased from farms known to be free of piroplasmosis. Initially a blood smear was taken from each calf, stained with Giemsa stain, and examined. All were negative for blood parasites.

Experimental infection

Calves were infected by the subcutaneous inoculation of 50-ml infective blood showing a minimum 10 per cent parasitaemia of a locally isolated virulent strain of *B. bigemina.* This strain had been maintained by succesive passage through splenectomized calves and stored at – 70°C.

Test drugs

The main compound under investigation was 3,3-bis- (2-imidazolin-2-yl) carbanilide dihydrochloride, as a solution containing 45 mg/ml administered by subcutaneous injection (Imidocarb dihydrochloride). For comparison of therapeutic activity *N,N*-(dimethylquinolylium-methylsulphate-6-)urea, as a solution containing 50 mg/ml was administered by subcutaneous injection.–

Observations

During each experiment calves were maintained under constant surveil-

lance. Each morning a clinical assessment and blood smear examination was carried out. Parasitaemia was assessed by counting the number of red blood cells cells parasitized on examination of 1000 such cells in a Giemsa-stained blood smear. The number infected was then-expressed as percentage, e.g. 10 cells parasitized in 1000 = 1 per cent parasitaemia. Rectal temperatures were recorded twice daily for every calf.

Treatments

All treatments were administered in the afternoon as considered appropriate to each experiment and with due consideration to the clinical assessment, rectal temperature, and parasitaemia on the morning blood smear.

In each experiment calves were observed for 1 month post-injection. In instances where reactions were recorded this period was extended until observable symptoms had ceased.

RESULTS

Experiment 1: *the therapeutic activity of Imidocarb dihydrochloride*

Twenty-four calves (20 *Friesian and* 4 *Sarabi*) were inoculated with infective *B. bigemina* blood as decribed (day 1).

Twenty-one of the calves (18 Friesian and 3 Sarabi) were treated with 1 mg/kg Imidocarb dihydrochloride at various stages of the infection (see Table 1) on an individual basis (days 4 to 7). When anaemia was evident, supportive therapy with vitamin B12, copper and cobalt was also administered.

Following treatment, the parasitaemia was arrested and within 48 h no parasites were seen in blood smears from treated calves. Clinical recovery was recorded in all cases.

The three untreated controls died of acute piroplasmosis (days 8 to 9) (see Table II).

Experiment 2: *the elimination of the carrier state and the development of resistance to re-infection* (*immunity*)

(*a*) Seven calves (6 Friesian and 1 Sarabi) were inoculated with infecte *B. bigemina* blood as described.

Imidocarb dihydrochloride was administered at 1 mg/kg to seven of the calves on an individual basis following development of a parasitaemia and

TABLE I

EXPERIMENT I. 21 CALVES TREATED WITH IMIDOCARB DIHYDROCHLORIDE AT A DOSE OF 1 MG/KG

	Pre-treatment		*Clinical symptoms*	*Blood-smear examination*
Calf no.	B. bigemina *parasitaemia* (%)	*Temperature* (°C)	*Anaemia* (A) ± *haemoglobinuria* (H)	*Days post-treatment to negative parasitaemia*
50/38	1	39	—	1
50/4007	1	40·2	—	1
51/88	1	39·5	—	1
51/111	2	39·6	—	1
51/12	2	39·6	—	1
51/38	2	39	—	1
50/19	4	39	—	1
50/48	4	41	—	1
50/37	6	39·2	—	1
51/46	6	40·9	A	1
50/5	10	40·9	—	1
51/34	10	39·5	—	1
51/36	10	40·9	A	1
51/51	10	40·4	A	1
51/6	11	40·6	—	2
51/43	13	40·2	—	1
50/47	14	41·6	—	1
51/47	14	40·7	—	1
51/49	14	40·9	A	2
51/52	25	41·3	A + H	2
51/39	40	41·3	A + H	2

TABLE II

EXPERIMENT I. UNTREATED CONTROL CALVES (3)

Calf no.	*Maximum recorded parasitaemia* (%)	*Maximum recorded temperature*	*Clinical symptoms*	*Days post-infection to death*
51/48	50	42·0	A + H	8
50/38	68	41·6	A + H	8
50/9	70	41·9	A + H	9

A = anaemia; H = haemoglobinuria.

temperature rise. While all treated calves recovered as in experiment 1, the three untreated calves died of acute piroplasmosis.

Between 25 and 61 days after treatment the seven calves were splenectomized. No relapse of the infection was recorded and no parasites were seen in the blood smears during the period of 30 days post operation.

(*b*) Two further Friesian calves were inoculated with infective *B. bigemina* blood as described. At the time of treatment with Imidocarb dihydrochloride, at 1 mg/kg, four days post-inoculation, one calf showed a 15 per cent parasitaemia and had a temperature of 41.4°C, the second showed a 10 per cent parasitaemia and a temperature of 40.9°C. Both calves recovered and were kept under observation.

At 60 days post-treatment 100 ml of blood was collected from each of the treated calves. In each case this was injected into a healthy, intact, susceptible calf. In neither of the healthy susceptible calves was there any evidence of *B. bigemina* infection over 30 days post-inoculaton.

At 100 days post-treatment the original calves each received a further inoculation of infective *B. bigemina* blood. A 1 per cent parasitaemia was recorded in blood smears from each of the two calves at 10 days and 20 days respectively. There was no accompanying rise in temperature and no clinical evidence of the infection. The piroplasms disappeared from the blood cells over a period of 72 h in both cases without any treatment.

Experiment 3: the margin of safety of Imodicarb dihydrochloride

Two groups of calves were treated with Imidocarb dihydrochloride at ten and five times the therapeutic dose.

Group I. Four calves (2 Friesian and 2 Sarabi) were treated with 10 mg/kg Imidocarb dihydrochloride by subcutaneous injection. Within 10 min all four calves exhibited profuse salivation. Slight muscle fasciculation with some dysponea also developed within 1–2 h of the injection. These symptoms sub sided in a period of 6 h in three of the calves, all of which made a complete and uneventful recovery. In the fourth calf, although the above syptoms subsided within 24 h, thereafter the calf showed a reduced appetite and progressive dullness until it died on the twelfth day post-injection.

Group 2. Eleven Friesian calves were treated with 5 mg/kg Imidocarb dihydrochloride. There were no observable toxic effects in six of the calves.

In the remaining five calves transient symptoms of salivation, slight muscle fasciculation and dyspnoea were observed. These had subsided within 3 h of the injection and thereafter all the calves returned to normal.

A mild local reaction was observed at both 10 mg/kg and 5 mg/kg as a slight to moderate oedematous swelling at the site of injection. The swelling dis appeared within 72 h.

Experiment 4: the comparison of the therapeutic activity of Imidocarb dihydrohloride and Acaprin

Fourteen Friesian calves were infected with *B. bigemina* as described. Two to three days after the first appearance of a parasitaemia, when a pyrexia was recorded, seven calves were treated with I mg/kg Imidocarb dihydrochloride and seven with I mg/kg Acaprin (see Table III),.

TABLE III

THE COMPARISON OF THE THERAPEUTIC ACTIVITY OF IMIDOCARB DIHYDROCHLORIDE AND ACAPRIN

	Pre-treatment			
Calf no.	*Parasitaemia* (%)	*Temperature* (°C)	*Clinical toxicity*	*Reappearance of parasitaemia: days post-treatment*
Imidocarb dihydrochloride				
51/131	6	40·3	—	—
52/5	8	40·1	—	—
52/6	10	40·9	—	—
52/3	11	40·5	—	—
52/109	11	41·0	—	—
51/110	12	40·8	—	—
52/4	16	40·8	—	—
Acaprin				
51/95	4	40·4	+	—
51/91	6	40·4	+	—
50/24	8	40·8	—	12
52/96	10	40·8	+	—
51/115	10	40·9	—	19
51/92	12	40·9	+	—
51/113	15	41·3	—	12

All the calves treated with Imidocarb dihydrochloride recovered. There was no evidence of toxicity and no relapse of the infection during the period of observation in these calves.

Four of the seven calves treated with Acaprin showed evidence of toxicity comprising salivation, muscle fasciculation and dysponoea. These symtoms subsided within 3 h of the injection.

A relapse of infection 12 to 19 days after the first treatment was also recorded in three of the calves that had been treated with Acaprin. A second injection of 0.5 mg/kg Acaprin was administered and a clinical recovery recorded.

DISCUSSION

The results of the experiments reported indicate that Imidocarb dihydrochloride administered by subcutaneous injection at a dose rate of 1 mg/kg is a

safe and highly effective therapeutic for the treatment of *B. bigemina* infection of calves, under Iranian conditions. It has been shown to offer distinct advantages over the medicament currently available in Iran both in terms of safety and efficacy. The therapeutic dose of 1 mg/kg was well-tolerated even in calves with severe and acute *B.bigemina* infection. Higher doses, 5-10 mg/kg, administered to healthy calves produced symptoms suggestive of an anticholinesterase activity, the severity of which appears to be dose-related.

An important result of the second experiment is a resitance to re-infection that was demonstrated in calves challenged 100 days after treatment with 1 mg/kg Imidocarb dihydrochloride on day 4 of a primary infection. As this dose level had been shown to sterlize *B. bigemina* infection, the "immunity" persisted in the absence of blood parasites. This finding would support the suggestion by Hart *et al.*, 1971), that the immunity following sterilization of a primary infection of short duration is by no means negligible. Certainly in this instance although both calves developed a very low-grade parasitaemia (1 per cent), they remained clinically normal and were refractory to this second infection.

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Etudes sur le dihychloride carbimide dans l'infection expérimentale par *Babesia bigemina* chez le veau

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Résumé. On décrit quatre essais chimiothérapiques en Iran avec le babesicide 3,3-bis (2–imidazoline-2-yl) dihydrochloride carbanilide (dihydrochloride carbimide). L'administration subcutanée d'une dose de I mg/kg s'avéra très efficace dans le contrôle de l'infection expérimentale par une espèce virulente locale de *Boabesia bigemia* chez le veau. Alors que les veaux triatés étaient en apparence stérilizés par rapport à l'infection. ils se montrèrent au bout de 100 jours résistants à une réinfection clinique en dépit de l'apparition de parasitémies secondaires.

On n'observa aucun effet toxique que l'on pût attribuer à l'administration de la dose thérapeutique de I mg/kg de dihydrochloride carbimide. Seuls des effets minerus et passagers furent observés pour 5 mg/kg et 10 mg/kg; un veau parmi quatre mourut 12 jours après l'injection. On considéra que le composé possédait un indes thérapeutique satisfaisant et ils s'avéra moins toxique et plus efficace dans le traitement de *B. bigemina* que l urée-*N,N*- (dimethylquinolylium-methylsulfate-6) (acaprine) employée couramment en Iran.