

**STUDIES ON IMIDOCARB DIHYDROCHLORIDE  
IN EXPERIMENTAL *BABESIA OVIS*  
INFECTION IN SPLENECTOMIZED LAMBS (\*)**

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SUMMARY

The therapeutic activity of 3,3-bis-(2-imidazolin-2-yl) carbanilide dihydrochloride (imidocarb dihydrochloride), in doses from 1 to 6 mg/kg bodyweight, was evaluated against experimental infection with a virulent strain of *Babesia ovis* in sheep.

The administration of imidocarb 2HCl at less than 6 mg/kg bodyweight was found to be ineffective in eliminating the parasites, and relapses occurred. Subcutaneous administration of the drug in divided doses of 2 mg/kg for three days proved to be highly effective and no relapses occurred among the 27 treated splenectomized lambs.

Subcutaneous inoculation of blood from these treated lambs into clean, splenectomized lambs indicated complete elimination of the parasites. Symptoms suggestive of toxicity of the drug were observed following a single injection of 10mg /kg bodyweight, but not after repeated lower dosages.

In a comparative study, N,N-(dimethylquinolylium-methylsulphate-6-) urea (quinuronium sulphate), at a dose of 1 mg/kg was ineffective in eliminating *B. ovis*. The margin of safety of quinuronium sulphate was small, toxic effects being observed with doses of 1 mg/kg bodyweight, even in divided doses of 0.5 mg/kg/day.

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## INTRODUCTION

Various carbanilide compounds were shown, by Schmidt, Hirt & Fischer (1969), to have activity against *Babesia rodhaini* in mice. Beveridge (1969) confirmed these results and reported imidocarb 2HCl as the most active babesiacidal drug in this series. Brown & Berger (1970), Callow & McGregor (1970) and Hashemi-Fesharki (1975) have demonstrated the activity of imidocarb 2HCl against experimental *Babesia bigemina* infection in cattle. Imidocarb 2HCl was also shown, by Frerichs, Allens & Holbrook (1973), to be highly effective against *B. equi*.

*B. ovis* infection in sheep is fairly prevalent in Iran. Experiments were therefore designed to test imidocarb 2HCl against *B. ovis* infection in splenectomized lambs.

This paper records the results of these trials. The therapeutic activity of imidocarb 2 HCl was also compared with quinuronium sulphate (Acaprin; Bayer Ltd.), which is currently used for the treatment of piroplasmosis in Iran.

## MATERIALS AND METHODS

### *Experimental animals*

Splenectomized lambs of local breed aged three to eight months were used. They were housed in tick-free stables. Before commencement of the experiment, blood smears of all lambs were examined to ensure that they were free from protozoan infections.

### *Infection*

The lambs were infected by subcutaneous (s.c.) or intravenous (i.v.) inoculation of 30 ml infective blood showing a minimum 5 to 10% parasitaemia of a locally isolated virulent strain of *B. ovis*. This strain had been maintained by passage in splenectomized lambs and preserved at -70°C.

### *Test drugs*

The main compound under test was 3,3-bis-(2-imidazolyl) carbanilide dihydrochloride (imidocarb dihydrochloride), as an aqueous solution containing 4.5% of the drug. For comparison of therapeutic activity, N,N-(dimethylquinolylum-methylsulphate-6)-urea (quinuronium sulphate) was used as a solution containing 20 mg/ml. Both drugs were administered subcutaneously.

## *Observations*

The temperatures of all lambs were recorded each morning and evening. Blood smears obtained from the ear vein were examined after staining with Giemsa's stain. The percentage of red blood cells parasitized was determined by the examination of 1000 cells. In most cases treatment was carried out on the day on which animals exhibited maximum parasitaemia. Animals were kept under close observation for one to two months after infection.

## RESULTS

### *Experiment 1. Effect of a single dose of imidocarb dihydrochloride*

Twenty-one splenectomized lambs received blood from a lamb infected with *B. ovis*, either fresh from the donor or after storage at  $-70^{\circ}\text{C}$ . Fourteen lambs (Group A) were treated with a single injection of imidocarb 2HCl. Seven of them received 1 mg/kg, and seven received 2 mg/kg.

Seven lambs (Group B) received 4 mg/kg of drug in equally divided doses of 2 mg/kg/day on consecutive days.

Relapse occurred in four lambs in Group A and two lambs in Group B, but blood smears became negative after a further injection of drug at 2 mg/kg.

### *Experiment 2. Effect of repeated doses of imidocarb 2HCl*

Twenty-seven splenectomized lambs were infected with *B. ovis* and 25 of them developed patent parasitaemia with maximum parasitaemia ranging from 0.6 to 22%. They were treated with 6 mg/kg imidocarb 2HCl in three equally divided doses at 24 h intervals. In 22 of the 27 lambs the parasite could not be found in blood smears one to two days after the first injection of drug, although in three lambs parasites were seen for one to two days after the third injection. In the two lambs in which no parasitic reaction was observed, the thermal reaction disappeared one to two days after the third injection of drug. The results are summarized in Table I.

### *Experiment 3. Elimination of infection with imidocarb 2HCl*

Two splenectomized lambs were each inoculated i.v. with 50 ml of blood from two lambs in Experiment 2 one month after their infection (25 days after treatment). Neither parasitic nor thermal reaction were observed during the subsequent one month observation period.

Two months after infection 100 ml of blood was taken from each of four lambs treated in Experiment 2 and was inoculated s.c. into four splenectomized lambs. They were observed for one month and during this time no parasitic

or thermal reaction was seen. Two of these lambs were then challenged with the same strain of *B. ovis*. They developed typical parasitic and thermal reactions and maximum parasitaemia reached 3% on the fifth days of infection in both cases.

TABLE I  
EFFECT OF THREE CONSECUTIVE DAILY INJECTIONS OF IMIDOCARB 2HCl AT A DOSE OF 2 MG/KG/DAY ON *B. OVIS* INFECTION

<i>Lamb No.</i>	<i>Pre-treatment B. ovis parasitaemia*</i>	<i>Pre-treatment temperature</i>	<i>Negative parasitaemia—days after first injection</i>
3	—	41.3	—
4	—	39.9	—
5	30	41.5	2
6	70	42.0	2
7	90	41.8	2
8	10	41.3	1
29	50	40.4	1
38	40	42.0	1
41	30	42.0	3
42	30	42.0	1
60	30	42.0	1
61	20	42.0	1
64	6	41.6	1
72	70	41.5	1
76	25	41.3	2
91	60	41.2	1
92	40	42.0	2
92	35	41.4	2
93	90	40.8	2
95	60	41.9	2
121	60	42.0	2
123	8	42.0	1
124	220	42.0	3
125	45	41.4	2
127	50	42.0	4
128	110	41.6	2
131	75	41.8	2

\* Expressed as number of infected cells per 1000 RBC's.

#### *Experiment 4. Toxicity of imidocarb 2HCl*

Two healthy lambs were injected with a single dose of 10 mg/kg imidocarb 2HCl s.c. They exhibited a marked reaction, manifested by salivation, muscle fasciculation and dyspnoea. The reaction commenced within 10 to 15 min after injection and lasted for one hour.

One healthy lamb was injected with 6 mg/kg imidocarb 2HCl s.c. in equally divided doses of 2 mg/kg for three days. Thirty days after the last injection this animal was again injected with 2 mg/kg/day s.c. for three days. No reaction suggestive of toxicity of the drug was seen.

*Experiment 5. Effect of single and repeated doses of quinuronium sulphate*

Seven splenectomized lambs were infected with fresh blood or with blood stored at  $-70^{\circ}\text{C}$  and all developed parasitic and thermal reactions with maximum parasitaemia up to 20%. Three lambs were injected with 0.5 mg/kg of quinuronium sulphate for two days, three were given a single injection of 1 mg/kg of quinuronium sulphate, and one received 1 mg/kg/day for two days. Five out of seven lambs showed toxic reactions manifested by salivation, muscle fasciculation and dyspnoea. Relapse occurred in one lamb on the twenty-sixth day of infection although the parasitic reaction was mild and subsided on treatment with another injection of quinuronium sulphate at 0.5 mg/kg. The results are summarized in Table II.

TABLE II  
EFFECT OF SINGLE AND REPEATED DOSES OF QUINURONIUM SULPHATE ON *B. OVIS* INFECTION

Lamb No.	Pre-treatment parasitaemia*	Pre-treatment temperature ( $^{\circ}\text{C}$ )	Quinuronium sulphate mg/kg		Clinical toxicity	Re-appearance of parasitaemia – days post-treatment
			Day 1	Day 2		
70	10	42.0	1.0	—	+	—
71	90	42.0	1.0	1.0	+	—
75	4	39.7	1.0	—	+	—
121	8	41.8	1.0	—	+	—
122	200	42.0	0.5	0.5	—	—
125	200	42.0	0.5	0.5	+	—
126	200	42.0	0.5	0.5	—	22

\* Expressed as number of infected cells per 1000 RBC's.

*Experiment 6. Elimination of infection with quinuronium sulphate*

Two splenectomized lambs were injected i.v. with 50 ml of blood from lambs in Experiment 5 one month after infection with *B. ovis* and treatment with quinuronium sulphate at 1.0 mg/kg as a single dose, or two doses of 0.5 mg/kg. Both recipient lambs developed thermal and parasitic reactions after four to five days incubation period. Parasitaemia reached 20% and 4% respectively on the sixth day of infection. These two lambs were successfully treated with imidocarb 2HCl at 2 mg/kg/day for three days.

### Experiment 7. Control experiment

Four splenectomized lambs were infected with *B. ovis* as in the earlier experiments, but received no treatment. They died in six to seven days (Table III).

TABLE III  
*B. OVIS* INFECTION IN UNTREATED, SPLENECTOMIZED CONTROL LAMBS

Lamb No.	Maximum recorded parasitaemia*	Maximum recorded temperature (°C)	Days post-infection to death
135	500	42.0	6
141	450	42.0	6
142	435	41.9	7
143	475	41.8	7

\* Expressed as number of infected cells per 1000 RBC's.

## DISCUSSION

Experiment 1 suggests that a dose of 1 to 4 mg/kg of imidocarb 2 HCl is inadequate for preventing relapse of *B. ovis* infection in splenectomized lambs. In contrast to this (Experiment 2) none of the 27 splenectomized lambs treated for three days at a dose level of 2 mg/kg imidocarb 2HCl/day showed a relapse.

The results obtained in Experiment 3 are interesting since they indicate that *B. ovis* is completely eliminated from splenectomized lambs by the injection of three daily doses of 2 mg/kg imidocarb 2HCl.

The injection of imidocarb 2HCl at a dose level of 10 mg/kg caused toxic symptoms in healthy non-splenectomized lambs, but three daily doses of 2 mg/kg were well tolerated and were highly effective in eliminating *B. ovis* infection.

One out of seven animals treated with two daily injections of 0.5 mg/kg of quinuronium sulphate relapsed, and the parasites were not completely eliminated as shown by the transmission study. Five out of seven lambs treated with either two doses of 0.5 mg/kg or one dose of 1 mg/kg of quinuronium sulphate showed marked toxic reactions.

This study indicates that the use of quinuronium sulphate for the treatment of *B. ovis* infection at the recommended therapeutic dosage of 1 mg/kg is both ineffective and also toxic in a substantial proportion of treated lambs. An increase in dosage to achieve greater therapeutic efficiency is accompanied by increased toxicity.

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