CHEMOTHERAPY OF EXPERIMENTAL BABESIA BIGEMINA INFECTIONS WITH IMIDOCARB DIHYDROCHLORIDE

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SUMMARY

Chemotherapeutic trials with a new babesicide 3,3'-bis-(2-imidazolin-2-yl) carbanilide dihydrochloride (4A65) are reported. The drug was shown to be highly effective in the control of rising Babesia bigemina parasitaemias in experimentally infected splenectomized calves. Administered subcutaneously or intramuscularly at levels down to 1 mg/kg and intravenously at dose rates as low as 0.125 mg/kg imidocarb dihydrochloride suppressed both the multiplication of the parasite and the development of anaemia in acute infections. Toxicity observations indicated a high therapeutic index.

INTRODUCTION

Schmidt, Hilt & Fischer (1969) reported the activity of a new series of compounds synthesised in the laboratories of Dr A. Wander S.A., Berne, against Babesia rodhaini infections in mice. Beveridge (1969) subsequently compared the activity of a selected member of this series, 3,3'-bis-(2-imidazolin-2-yl) carbanilide dihydrochloride†, with that of other babesicides against B. rodhaini. She concluded that imidocarb dihydrochloride (4A65) was a highly effective babesicide at very low dose levels. Callow & McGregor (1970) have since shown that 4A65 was active against both B. argentina and B. bigemina in cattle.

This paper records the results of preliminary chemotherapeutic trials with 4A65 against B. bigemina infections in splenectomized calves. Toxicity and tolerance studies of this compound in calves and steers are described.

MATERIALS AND METHODS

The efficacy of the compound in controlling parasitaemia of B. bigemina and the concomitant anaemia normally resulting therefrom was evaluated in splenectomized calves undergoing acute reactions when infected with a blood passaged strain of this parasite. The babesicidal activity of 4A65 was compared with that of three commonly used babesicides. Toxicity trials were performed in splenectomized calves and in non-splenectomized adult steers.

Experimental animals.—Seventy-six splenectomized calves were infected with B. bigemina and used in the chemotherapeutic trials. These were high grade calves, mostly Ayrshire or Friesian, purchased at 1 to 3 weeks of age and splenectomized 2 to 3 weeks later. The

† Imidocarb dihydrochloride: Compound 4A65 (Burroughs Wellcome & Co. Ltd.)
majority of these were infected within four weeks of splenectomy, at which time they were free of blood parasites. One group of 25 comprised older calves which had been used in *Anaplasma marginale* chemotherapeutic trials and had been splenectomized 8 to 18 months previously.

For specific toxicity trials, 40 steers aged 30 to 50 months and an additional 32 splenectomized calves aged 1 to 15 months, were used.

All experimental animals were housed, and tick infestation was controlled by spraying with toxaphene and dioxathion* at 4 to 5 day intervals.

**Infections.**—Cattle were infected by the subcutaneous or intravenous inoculation of 5 ml blood patently infected with *B. bigemina* of the strain termed WRL 2035. This strain of Babesia was isolated from a naturally infected steer which reacted clinically. Sequestrene (di-sodium ethylene diamine tetra-acetate) 7 mg/ml was the anticoagulant used. The work described here was confined to passages 2 to 12 of this strain.

On two occasions the strain was frozen using an empirical technique based on that described by Barnett (1964). Five ml aliquots of infected blood in bijou bottles with 7-5 per cent glycerol as the cryoprotectant were slowly frozen to $-79^\circ$ C at a rate of approximately $1^\circ$ C/minute. These aliquots were stored at a temperature of $-60^\circ$ C for periods of up to six months. On resuscitation, the frozen material was thawed rapidly in a $37^\circ$ C waterbath and passaged once through a splenectomized calf before being used in a chemotherapeutic trial. Standard infection of recently splenectomized calves gave rise to a 1 per cent parasitaemia in a mean prepatent period of 2.5 days.

**Test drugs.**—The compound under test was 3,3'-bis-(2-imidazolin-2-yl) carbanilide, which has been given the generic name imidocarb. (British Veterinary Codex 1970). Both the base and the dihydrochloride were used in toxicity trials. The structural formula of the latter is given below.

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\begin{align*}
\text{\text{NH-CO-NH}} & - & \text{\text{NH}} \\
\text{\text{N \ N \ N}} & - & \text{\text{2 HC1}} \\
\text{\text{NH}} & - & \text{\text{N}}
\end{align*}
\]

Dose rates were calculated on actual amount of the salt used, 82 mg [base being equivalent to 100 mg dihydrochloride.

In both toxicity and chemotherapeutic trials, where high dose levels were administered, 4A65 was given as a 10 per cent solution in distilled water. At lower dose levels, concentrations down to 0.5 per cent were utilised. The base was used as an 0.5 per cent to 10 per cent suspension in distilled water, volumes of injections varying from 2.5 to 20 ml for convenience of handling and observation.

For comparative purposes three commonly used babesicides were tested for efficacy against *B. bigemina* infections in splenectomized calves. These were:

- Diminazene aceturate**
- Quinuronium sulphate†
- Amicarbalide di-isethionate‡

Test treatments were administered in the afternoon, approximately six hours after sampling for observations on parasitaemia and haematological status.

* Alik—Cooper, McDougall & Robertson (East Africa) Ltd.
** Berenil—Farbwerke Hoechst AG.
† Pirevan—Evans Medical Ltd.
‡ Diampron—May & Baker Ltd.