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AMICARBALIDE: A THERAPEUTIC AGENT FOR ANAPLASMOSIS

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ABSTRACT

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When administered subcutaneously in 2 equal daily doses at a total dosage rate of 20 mg/kg, amicarbalide was found to be an effective agent for controlling acute infections of *Anaplasma marginale* and *A. centrale* in intact and splenectomized cattle. Attempts to sterilize patent and latent *Anaplasma* infections, however, were unsuccessful. At total dosage rates of 40 mg/kg and higher, amicarbalide exhibited potent hepato- and nephrotoxic tendencies.

Résumé

L'AMICARBALIDE, UN AGENT THÉRAPEUTIQUE DE L'ANAPLASMOSE

En injections sous-cutanées à raison de deux par jour, les deux doses étant égales et atteignant un taux global de 20 *mg/kg, l'amicarbalide s'est révélée être un medicament efficace contre les infections aiguës à* Anaplasma marginale *et* A. centrale *chez des bovins tant entiers que splénectomisés. On n'a cependant pas réussi à stériliser les infections patentes et latentes à* Anaplasma. *Si l'on porte la dose à un taux global de* 40 *mg/kg et plus, l'amicarbalide manifeste de fortes tendances hépatotoxiques et néphrotoxiques.*

INTRODUCTION

Two derivatives of carbanilide, imidocarb* and amicarbalide**, are known to be potent babesiacides. In a review of various babesiacides, Joyner & Brocklesby (1973) indicated that imidocarb is at least as active against anaplasmosis as the tetracyclines. The literature, however, has little information about the possible chemotherapeutic value of amicarbalide against the disease. Shone, Wells & Waller (1961), using 20 mg/kg of this drug, observed no effect either on the course of the disease or on the number of parasites in an animal suffering from a post-splenectomy relapse of *A. marginale*. Uilenberg (1970), in uncontrolled experiments, detected a regression of parasitaemia in post-splenectomy *A. marginale* relapses of latently infected animals after amicarbalide treatment. Although he successfully controlled primary reactions in 2 out of 3 splenectomized animals, he apparently did not consider these results sufficiently convincing to pursue the matter further.

The purpose of this study was to assess the effect of amicarbalide against *Anaplasma* infections under controlled conditions both in intact and splenectomized cattle.

MATERIALS AND METHODS

Therapeutic effect in intact animals

Experimental animals

Six Jersey and 19 Afrikaner oxen and heifers were obtained from the Keetmanshoop district in South West Africa where the average annual rainfall is less then 200 mm and anaplasmosis is virtually unknown. At the commencement of this study, these animals were from 1–2 years of age with a mass ranging from 140–320 kg. The animals were divided into 6 groups (see below) in such a way that the average mass of each group was approximately the same, and that each group contained one Jersey ox.

Strain of A. marginale

The strain was isolated from a naturally infected case in 1972 and stored in liquid nitrogen with 10% dimethylsulphoxide as cryoprotectant until used. A susceptible splenectomized calf, born and raised at this Institute, was inoculated with this stabilate and, when a parasitaemia of 15% was reached, blood was collected in ACD and animals in each of the 6 groups injected intravenously with approximately 1×10^{10} parasitized erythrocytes.

Drugs

Amicarbalide isethionate B. Vet. C. 50% m/v was administered subcutaneously at dosage rates of 5 and 10 mg/kg.

For comparative purposes, one group was injected intravenously with oxytetracycline* at a dosage rate of 10 mg/kg.

Experimental design

The 25 animals were divided into the following 6 groups:

Group 1 Four animals treated with amicarbalide (5 mg/kg) once only
Group 2 Four animals treated with amicarbalide (5 mg/kg) daily for 2 days
Group 3 Five animals treated with amicarbalide (10 mg/kg) once only
Group 4 Five animals treated with amicarbalide (10 mg/kg) daily for 2 days
Group 5 Three animals treated with oxytetracycline (10 mg/kg) daily for 2 days
Group 6 Four animals used as untreated controls.

The criteria used to evaluate the response to therapy were the effects on the levels of parasitaemia (Lotze, 1947; Miller, 1956) and packed cell volume (PCV). Thin blood smears, prepared daily from peripheral blood for 17 days prior to and for 10 weeks after inoculation, were fixed in methanol, stained with 5% Giemsa for 20 min and examined for the presence of parasites. The levels of infection were expressed as a percentage of infected erythrocytes. PCV determinations were made daily in duplicate from jugular blood, using Clay Adams microhematocrit tubes and a Christ microfuge. The haematocrit values were allowed to stabilize for 17 days prior to inoculation (Gartner, Callow, Grazien & Pepper, 1969). After treatment, daily PCV determinations were continued for 6 weeks.

* Imizol, Burroughs Wellcome & Co.
** Diampron, May & Baker Ltd

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* Terramycin 100, Pfizer Laboratories (Pty) Ltd

Although the animals had been grouped, the reaction of each animal was evaluated individually. Treatment was started on the day (Day 0) that the PCV of each animal fell to 22% or below and the results of the mean PCV and parasitaemia of both treated and control animals are illustrated as from Day 0.

Sterilizing effect and toxicity

Experimental animals

Seven intact animals, infected with *A. marginale* 2 months prior to this and used as untreated controls or treated with tetracycline during the study described above, were used in an attempt to sterilize latent infections.

Experimental design

The animals were divided into 4 groups:

Group 7 Two animals treated (10 mg/kg) daily for 4 consecutive days

Group 8 Two animals treated (10 mg/kg) 4 times at 72 h intervals

Group 9 Two animals treated (10 mg/kg) 7 times at 72 h intervals

Group 10 One untreated carrier of *A. marginale*.

Four weeks after the last treatment 1 of the treated animals in Group 8 and the control animal (Group 10) were splenectomized in an attempt to induce relapses.

Pathology and histopathology

Post-mortem examinations were performed on the 2 animals in Group 9. One animal died on Day 27, while the other was destroyed *in extremis* on Day 28. In addition, portions of the liver, kidney, spleen, brain, heart muscle and lymph nodes were collected and fixed in 10% formalin. These were subsequently embedded in paraffin wax, sectioned at 4–6 μm and stained with haematoxylin-eosin stain.

Clinical pathology

Clinical pathological observations were carried out on the blood of 1 of the animals in Group 9 shortly before death, and both animals in Group 8, 2–3 weeks after the last treatment. The following determinations were done: glutamic oxaloacetic transaminase (GOT), gamma-glutamyl transpeptidase (γ-GT), bilirubin, total plasma proteins and serum urea nitrogen (SUN).

Therapeutic effect in splenectomized animals

Animals

Ten Hereford or Hereford cross cattle, born, raised and kept at this Institute under strict tick-free conditions, were used to determine the effect of amicarbalide on the primary reactions of *A. marginale* and *A. centrale* in splenectomized animals.

Strains of Anaplasma *spp.*

The strain of *A. marginale* was the one described above and the *A. centrale* strain that used for vaccine production which had been maintained by serial passage in splenectomized animals at this Institute for many years. Both strains produce fatal infections in untreated splenectomized animals.

Experimental design

The animals were divided into 3 groups:

Group 11 Four animals infected with *A. marginale* treated with amicarbalide (10 mg/kg) daily for 2 consecutive days

Group 12 Four animals infected with *A. centrale* treated with amicarbalide (10 mg/kg) daily for 2 consecutive days

Group 13 Two animals infected with *A. centrale* treated with amicarbalide (10 mg/kg) twice with a 48 h interval between treatments.

The only criterion used to evaluate the response to therapy was its effect on the level of parasitaemia (Lotze, 1947; Miller, 1956). Because of their value some of the animals were given supportive treatment to ensure recovery, thus precluding the use of the PCV as a parameter.

After the initial reaction had subsided, blood smears were examined daily for a further 60 days or more. If a rise in parasitaemia occurred, haematocrit levels were determined and the animals were retreated if necessary.

Statistical analysis

The data on parasitaemia and PCV of Groups 1–5 were compared with those of Group 6 (untreated controls) by calculating t-test probabilities. The lack of a sufficient number of control animals precluded the use of statistics in the other experiments.

Results and Conclusions

Therapeutic effect in intact animals

The strain of *A. marginale* used in this trial caused a marked reduction in the PCV's of the untreated controls, but was not sufficiently virulent to cause death.

The response of the PCV and parasitaemia to treatment is illustrated in Fig. 1.

Statistically significant reductions in the level of parasitaemia of the treated groups were first detectable on the following days:

Group 5 (tetracycline; 10 mg/kg; 2×): Day 2 ($P<0,05$)

Group 3 (amicarbalide; 10 mg/kg; 1×): Day 1 ($P<0,025$)

Group 4 (amicarbalide; 10 mg/kg; 2×): Day 2 ($P<0,005$)

Group 2 (amicarbalide; 5 mg/kg; 2×): Day 3 ($P<0,05$)

Group 1 (amicarbalide; 5 mg/kg; 1×): Day 3 ($P<0,025$)

Similarly, statistically significant differences between the PCV levels of treated and control groups were first detected on the following days:

Group 5 (tetracycline; 10 mg/kg; 2×): Day 3 ($P<0,05$)

Group 4 (amicarbalide; 10 mg/kg; 2×): Day 3 ($P<0,05$)

Group 3 (amicarbalide; 10 mg/kg; 1×): Day 4 ($P<0,05$)

The differences between Groups 1 and 2 (amicarbalide; 5 mg/kg 1× & 2×) and the control animals (Group 6) were not significant.

Compared with that in the controls (Group 6), a statistically significant reduction in the time that the PCV was below 22% was observed in the following groups (Fig. 1):

Group 5 (tetracycline; 10 mg/kg; 2×): $P<0,005$

Group 4 (amicarbalide; 10 mg/kg; 2×): $P<0,005$

Group 3 (amicarbalide; 10 mg/kg; 1×): $P<0,05$

Once again the differences between the animals in Groups 1 and 2 (amicarbalide; 5 mg/kg; 1× and 2×) and the control animals (Group 6) were not significant.

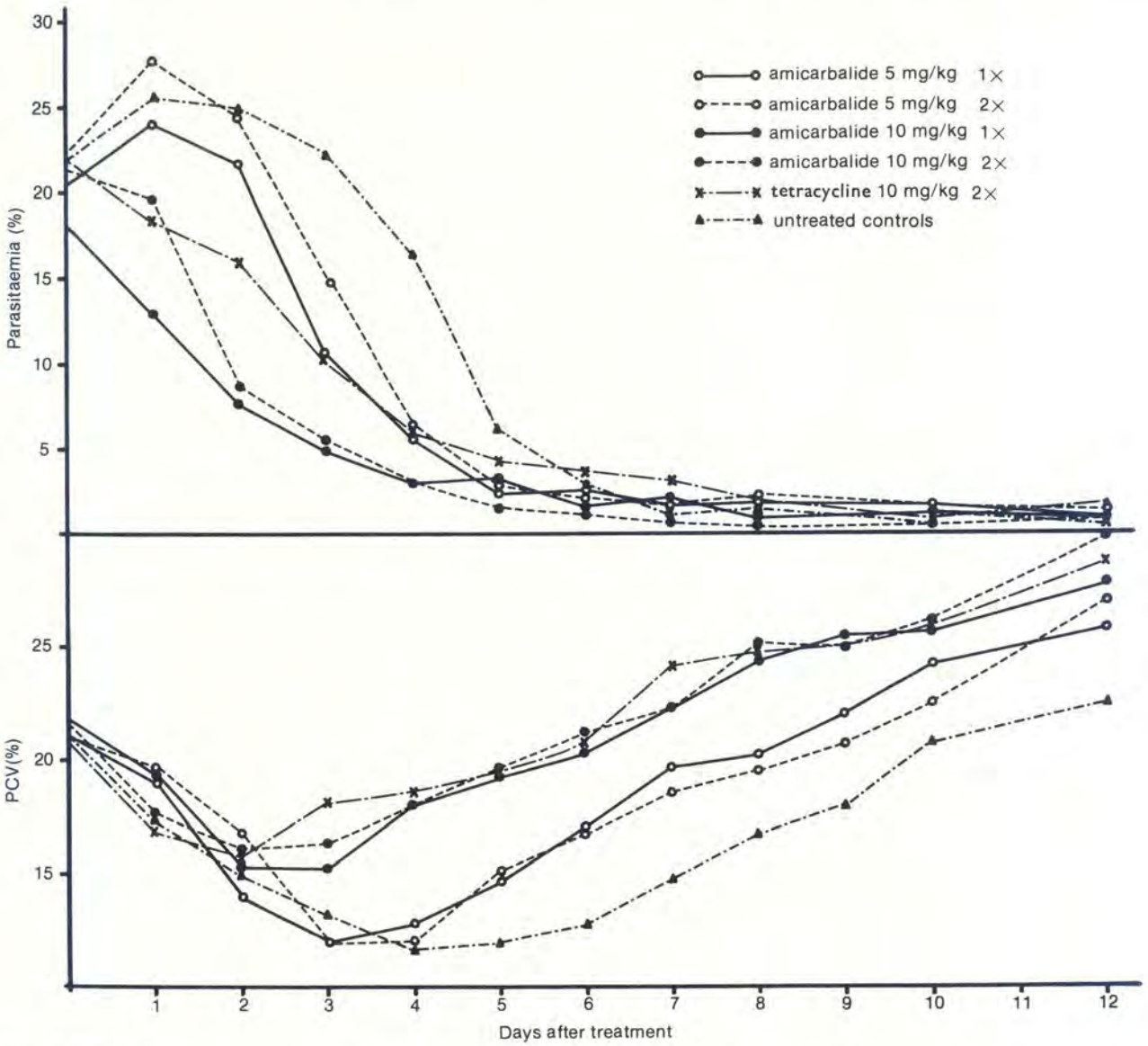

FIG. 1 Effect of treatment on *A. marginale* parasitaemia and packed cell volume in intact animals

From these results it is evident that amicarbalide injected once or twice at a dosage rate of 10 mg/kg s/c had a therapeutic effect comparable with that of oxytetracycline administered twice at 10 mg/kg intravenously on *A. marginale* infections in intact animals.

Post-therapy relapses in intact animals

The recurrence of the parasites in blood smears of the animals after treatment at the dosages mentioned above as well as the resultant drop in PCV are presented in Table 1.

After the initial reaction, 2 characteristics of the parasitaemia were observed (Table 1), namely, the mean maximum relapse parasitaemia (calculated from the peak relapse parasitaemias of each animal in every group) and the interval between primary reaction and relapse (the mean interval between the 1st day of treatment and the day of peak relapse parasitaemia).

It is evident from Table 1 that the different dosages of amicarbalide did not affect the severity of the subsequent relapses or the interval between the initial reaction and relapse. The results of these groups, when compared with those of groups treated either with tetracycline or left untreated, are similar, except that treatment with tetracycline resulted in a prolonged interval between treatment and the peak of the parasitic relapse.

Therapeutic effect in splenectomized cattle

The effect of therapy on *A. marginale* and *A. centrale* parasitaemia is illustrated in Fig. 2.

At a dosage level of 10 mg/kg repeated after 24 h, amicarbalide had a rapid effect on the parasitaemia of both *A. marginale* and *A. centrale*. If administration of the second dose was delayed for 48 h, however, the decline of *A. centrale* parasitaemia on the first day after treatment was followed by an increase on the second day (Fig. 2). This method of treatment was not attempted with *A. marginale* infections. Although no attempts were made to treat splenectomized animals with only one dose, these results indicate that a single treatment may be inadequate to control severe reactions in splenectomized animals.

TABLE 1 Relapses of *A. marginale* infections in intact animals after treatment with amicarbalide or tetracycline

Group No.	Treatment			Mean maximum primary parasitaemia (%)*	Mean maximum relapse parasitaemia (%)	Intervals (days)**	Mean minimum relapse PCV (%)
		Dosage mg/kg	No. of treatments				
1.....	Amicarbalide.....	5	1	20,3	10,0	27,0	26,0
2.....	Amicarbalide.....	5	2	22,5	10,7	26,0	24,7
3.....	Amicarbalide.....	10	1	18,0	9,3	26,2	26,5
4.....	Amicarbalide.....	10	2	21,2	13,0	30,5	22,8
5.....	Tetracycline.....	10	2	22,0	10,3	39,3	24,7
6.....	Untreated.....	—	—	22,0	10,5	31,8	25,0

* On first day of treatment
** Days after treatment of initial reaction

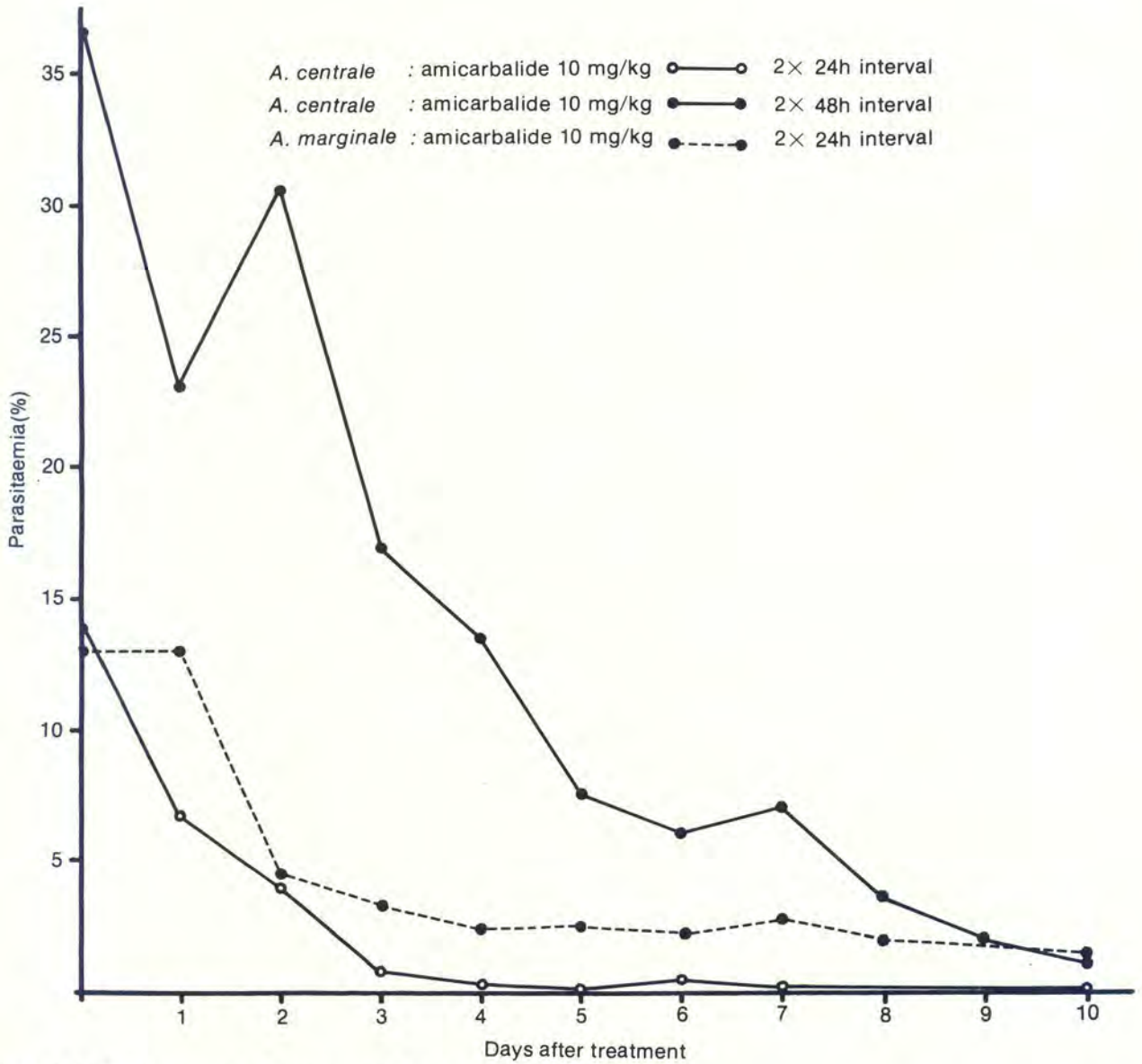

FIG. 2 Effect of treatment on parasitaemia in splenectomized animals

Post-therapy relapses in splenectomized animals

Relapses 23–48 days after treatment were a constant finding in the 10 splenectomized animals treated during the course of this study (Table 2). Of these, 8 required re-treatment. This phenomenon is by no means uncommon, however, and has, for example, been recorded after imidocarb treatment of *A. marginale* infections in splenectomized cattle (Joyner & Brocklesby, 1973). Similar observations have also been made at this Institute after the use of tetracycline (P. R. Barrowman, unpublished observations).

TABLE 2 Relapses of *A. marginale* and *A. centrale* infections in splenectomized animals after treatment with amicarbalide (2×10 mg/kg)

Group No.	Bovine No.	Parasite	Primary parasitaemia (%)*	Maximum relapse parasitaemia (%)	Interval (Days)***
11	1	*A. marginale*.....	28	20**	27***
	2		20	25**	23
	3		6	10**	33
	4		4	18	31
12	5	*A. centrale*.....	8	28**	37
	6		5	25**	32
	7		28	15**	46
	8		20	14	29
13	9	*A. centrale*.....	34	3	36
	10		39	9**	48

* On first day of treatment
** Animal treated to control reaction
*** Days after treatment of initial reaction

Sterilizing effect

Observations on this aspect of the treatment were limited on account of the toxic effects of repeated dosis of amicarbalide (see below). A total dose of 20 mg/kg, divided into 2 equal daily doses and given during the acute phase of the disease, failed to sterilize *A. marginale* infections in 5/5 intact and 4/4 splenectomized animals. Parasites persisted at microscopically detectable levels after treatment for the entire period of observation.

Amicarbalide at a dosage rate of 10 mg/kg given 4 times at 72 h intervals likewise failed to sterilize the infection in 2 latently infected, non-splenectomized cattle. No parasites were seen in the blood smears of one animal for 22 days and of the other for 28 days after the last treatment. Thereafter, parasites reappeared in readily detectable numbers. The latter animal and the untreated control were splenectomized on Day 28 after treatment and both developed clinical relapses.

Despite the fact that this drug had a noticeable anaplasmacidal effect when used in acute cases, it failed to eliminate the parasite altogether. This was achieved, however, with imidocarb at a dosage rate of 5 mg/kg given twice at an interval of 14 days (Roby & Mazzola, 1972). Kuttler (1971) likewise eliminated latent infections with levels of 4 and 6 mg/kg given 3 times at 24 h intervals.

Toxicity

A total dosage rate of 40 mg/kg given in 4 divided doses over 4 days (Group 7) caused the death of both treated animals 11–12 days after the last injection. Similarly, a total dose of 70 mg/kg given in 7 divided doses over a period of 18 days (Group 9) had a fatal outcome in 2/2 cattle (1 animal was killed *in extremis*) 3 days after the termination of treatment. Two animals given 40 mg/kg in 4 divided doses over a period of 9 days (Group 8) survived, however, but showed signs of hepatic and renal involvement (see below).

Gross pathology

At autopsy, the 2 animals examined (Group 9) appeared emaciated and showed a severe ascites, moderate hydrothorax and hydropericardium. A marked oedema of the abomasal folds and the mesentery, mainly between the loops of the proximal colon, was a striking feature in both cases. In 1 animal congestion and scattered petechial and ecchymotic haemorrhages were seen in the abomasal mucosa.

The liver in both cases was moderately enlarged, with a distinct yellow-brown to dark-brown lobulation and, in 1 case, the surface appeared uneven. The kidneys were enlarged and congested. Numerous foci, ranging in colour from white to light-grey and varying in size from pinpoint to 2–3 mm in diameter, were seen throughout the renal cortex and medulla.

The only other lesion observed at autopsy was an oedema of the gall-bladder wall and lungs.

Histopathology

Liver lesions of the sacrificed animal were acute and showed a severe centrilobular coagulative and lytic necrosis of the parenchyma with neutrophil infiltration. In the other case, necrosis was less pronounced and regeneration was already in progress. Enlarged and vesicular hepatocyte nuclei, mitotic figures and hepatocytes with 2–3 nuclei were seen in the latter as well as a moderate Kupffer cell proliferation and a slight portal reaction. Both livers showed cloudy swelling as well as hydropic and slight fatty degeneration.

Histopathological changes in the kidneys ranged from acute to chronic. Cloudy swelling and hydropic degeneration, affecting mainly the proximal convoluted tubules, and disseminated foci of mono-nuclear cell infiltration and fibrosis were observed. Other changes observed in the kidneys were: (i) dilatation of the tubules and capsule of Bowman, (ii) mineralized foci in the cortex and medulla, (iii) hypertrophy and hyperplasia of the tubular epithelium, mainly in the cortex, and (iv) hyalin and cellular tubular casts.

Lymphoid atrophy and slight haemosiderosis in the spleen and lymph nodes were the only noteworthy lesions seen in other organs.

Clinical pathology

The most constant chemical pathological change was an elevation of the activity of γ-GT (30–50 mIU/mℓ), detected in the sera of all 3 animals examined. This change, together with an increase in GOT activity (212 King units) in one of the animals, was indicative of liver pathology.

Renal disfunction was indicated by abnormally high SUN levels (41–130 mg/100 mℓ) in 2 of the animals.

Apart from the references below, the literature has very little information on these hepato- and nephrotoxic effects of amicarbalide in bovines. Pipano, Weisman, Raz & Klinger (1972) administered 4 consecutive daily doses of 10 mg/kg s/c to 5 animals but apparently did not notice the ill effects that were observed in this study when the same regimen of treatment was followed. Apart from mild transient ataxia, Ashley, Berg & Lucas (1960) likewise did not observe any systemic effects in calves that received a single s/c dose of 40 mg/kg. Taylor, Simpson & Martin (1972), however, did observe both gross and histologic lesions and serum enzyme changes indicating hepato- and nephro toxicity in equines after intramuscular administration of high doses of amicarbalide. These were especially noticeable when total amounts in excess of 35 mg/kg were used. Indications of some liver damage were seen after doses totalling as little as 17,6 mg/kg.

In conclusion, it is evident therefore that amicarbalide has not only babesicidal but also anaplasmacidal properties similar to those of imidocarb. Further study of the toxic effects of amicarbalide is needed, however, to establish its safety at therapeutic levels.

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