Brit. J. Pharmacol. (1954), 9, 37.

THE CHEMOTHERAPEUTIC ACTION OF PHENANTHRIDINE COMPOUNDS

PART VII

THE EFFECT OF 2 : 7-DIAMINO-9-α-THIENYL-10-METHYLPHENANTHRIDINIUM CHLORIDE 621C47) UPON INFECTIONS WITH *TRYPANOSOMA CONGOLENSE* AND *T. VIVAX* IN CATTLE*

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(RECEIVED AUGUST 14, 1953)

In a previous paper Brownlee, Goss, Goodwin, Woodbine and Walls (1950) described a number of new phenanthridinium compounds which were as active as dimidium against laboratory infections of *Trypanosoma congolense* in mice. Four of these compounds were subsequently given trials in cattle in Northern Nigeria (Goodwin and Unsworth, 1952; Goodwin and Chandler, 1952). One compound (150C47) was given an extended trial and the results showed that it was too toxic for use; another (621C47) eradicated the trypanosome infection and proved relatively non-toxic.

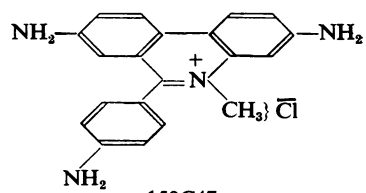
As the number of cattle used in the Nigerian experiments with 621C47 was very small, a further trial with this drug was carried out in the Sudan. A few experiments were also conducted with 150C47. A preliminary report of these experiments has been published by Evans (1952). The present paper gives the results in full.

MATERIAL AND METHODS

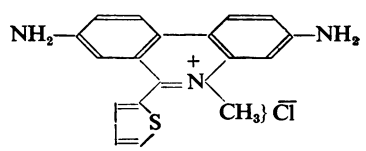
The structural formulae of 150C47 (2:7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride) and 621C47 (2:7-diamino-9-α-thienyl-10-methylphenanthridinium chloride) are shown in the next column. For comparison with these, the structural formula of dimidium is also given.

It should be noted that the bromide of 621C47, used in Nigeria by Goodwin, is less soluble than the chloride used in the present experiments. The drugs 150C47 and 621C47 were dissolved in water to give 2% w/v solutions, and doses were given subcutaneously behind the shoulder. Dimidium was given as a 1% solution and, for comparison, antrycide methylsulphate as a 5% solution—both by the subcutaneous route.

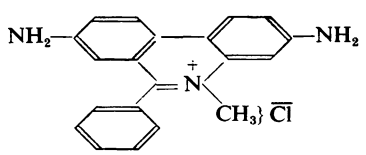
The experiments fall into two groups according to their geographical location, those carried out at Khartoum and those at Malakal (situated on the White Nile about 450 miles south of Khartoum). As the environmental conditions differed considerably, the two sets of experiments are described separately.



150C47



621C47



Dimidium

Khartoum Experiments

The animals used were bull calves, 1 to 2 years old, of the Zebu type commonly found in the Northern Sudan. Each calf was given a serial number and tethered to a stake. All were fed twice daily with freshly cut clover and unripe "*durra*" (*Sorghum*): water was also given twice daily.

The strains of *T. congolense* and *T. vivax* were originally obtained from natural infections observed in

* This work was done while R. A. Neal was working as a guest at the Veterinary Research Laboratories, Khartoum.

cattle at Malakal, and had since been transmitted by syringe. At the time of the first experiments the strain of *T. congolense* had been passaged six times in mice and twice in cattle, and that of *T. vivax* three times in cattle. In subsequent experiments the trypanosomes were drawn from positive controls of the preceding experiments. The animals were infected by subcutaneous injection of 5 ml. of infected blood.

Drug-resistant strains of *T. congolense* were obtained by treating the infected calf with sub-curative doses and, after it had relapsed, re-treating it with slightly larger amounts of drug. In this manner, two strains of *T. congolense* were obtained, one resistant to treatment with 5 mg./kg. of antrycide methylsulphate, the other resistant to 1.5 mg./kg. of dimidium.

The presence or absence of trypanosomes was determined by examining a drop of the ear blood. When it was impossible to examine fresh blood, dry films were stained with Field's stain and examined at a more convenient time. One hundred microscopic fields were examined (1/6 in. objective and 2 × ocular) before a blood-film was recorded as negative.

The blood of cattle inoculated with trypanosomes was examined daily until positive; the animals were then divided into groups and treated with the drugs. After treatment the ear blood was examined microscopically for trypanosomes as often as possible. The exact number of such examinations is recorded for each experiment.

Punch biopsies of the liver were taken in order to observe the effect of the phenanthridinium drugs upon that organ. The tissue was fixed in formol-saline. Frozen sections were cut, and stained with Sudan III.

The advantage of the experiments conducted at Khartoum is the absence there of any natural transmission of *T. congolense* or *T. vivax* by insect vectors; hence relapses after treatment could be attributed to failure of the drug treatment. As a precaution against the remote possibility of mechanical transmission of *T. congolense* or *T. vivax* by biting flies, all the cattle were sprayed with "gammexane" suspension twice weekly.

Malakal Experiments

The main difference between the experiments conducted at Malakal and those at Khartoum is that at Malakal the cattle were turned out to graze in the bush and, as the experiments were carried out during the dry season (February to April), there was very little green grazing except on the banks of the river. These conditions of poor nutrition and low water intake would be likely to reveal the toxic side-effects of a drug.

Since previous experiments in Nigeria (Goodwin and Chandler, 1952), and our experiments at Khartoum, had shown that 150C47 was too toxic for use only 621C47 was tried in the Malakal experiments.

Two groups of 30 cattle (1 to 3 year old Zebu bull calves, purchased locally) were inoculated with infected blood from naturally infected cattle—one group with *T. congolense*, the other with *T. vivax*. In addition, 6 calves found spontaneously infected with *T. vivax* were included in the group artificially infected with *T. vivax*. Not all the cattle inoculated became infected, but all were treated with 621C47.

The dose of drug used was 2 mg./kg., as the Khartoum work showed that doses lower than this were ineffective against *T. vivax*. Liver punch biopsies were taken from a few animals.

In the vicinity of Malakal no tsetse flies have been observed, but *T. vivax* and *T. congolense* are transmitted mechanically by blood-sucking flies. However, the experiments were carried out at the end of the dry season when the number of these flies is at a minimum.

As a further precaution against re-infections being confused with relapses, a small experiment was carried out to determine if 621C47 showed any prophylactic properties. A group of 50 calves was treated with 2 mg./kg. of 621C47. At weekly intervals after dosing, 5 of these animals were challenged with an inoculation of blood from a calf infected with *T. congolense*, and a further 5 were challenged with *T. vivax*. Two normal animals were also inoculated with the infected blood to act as controls at each challenge. After challenging, all cattle were examined on three successive days each week for 9 weeks.

Results

Therapeutic Experiments at Khartoum

The therapeutic effects of 621C47 and 150C47 upon *Trypanosoma vivax* and *T. congolense* are shown in Table I.

TABLE I

EFFECT OF 621C47 AND 150C47 UPON *TRYPANOSOMA CONGOLENSE* AND *T. VIVAX* INFECTION IN CATTLE

Drug	Dose mg./kg.	Cattle Infected with *T. vivax*		Cattle Infected with *T. congolense*	
		Ratio of Animals Cured to Animals Infected	Period Observed (Months)	Ratio of Animals Cured to Animals Infected	Period Observed (Months)
621C47	0·5	7/9	2·5	5/6	3
	1·0	4/8	2·5	6/6	3
	2·0	5/5	3	5/5	3
	4·0	—	—	4/4	3
—	Controls	0/6	3	0/4	0·5
150C47	0·5	0/5	4	4/6	4
	1·0	1/5	4	6/6	4
	2·0	2/2	4	6/6	4
—	Controls	0/4	4	0/7	4

These results show that a minimum dose of 2.0 mg./kg. of either 621C47 or 150C47 is required to cure all cattle infected with *T. vivax*. The animals began to relapse 13 days after treatment with 150C47 and 28 days after treatment with 621C47. In the blood of 2 of the 4 animals which relapsed after treatment with 1.0 mg./kg. of 621C47, trypanosomes were observed on 2 occasions only, but more frequently in the blood of the other 2 calves.

When it became apparent that doses of 1.0 and 0.5 mg./kg. of 621C47 were not sufficient to prevent relapses, the blood examination of these groups was stopped but the examination of those treated with 2 mg./kg. was continued. The blood of each calf in this group was examined 78 times over a period of 92 days without finding a trypanosome. Three of the infected, but untreated, calves died of acute trypanosomiasis; the others, however, survived, though trypanosomes were frequently observed in the blood up to the end of the experiment.

It should be noted that the results of treatment of *T. vivax* with 621C47 given in Table I are composite figures from three separate experiments. As they were carried out under similar conditions and gave similar results, they are treated here as one experiment.

Both of the phenanthridinium drugs were more effective against *T. congolense* than against *T. vivax*. In experiments with *T. congolense* a dose of 1.0 mg./kg. successfully eradicated the infection. Trypanosomes appeared in the blood of one calf 32 days after treatment with 0.5 mg./kg. of 621C47, and it was re-treated (30 days after observation of the relapse) with 1.0 mg./kg. of 621C47. This animal again relapsed 25 days after re-treatment; it was then given 1.5 mg./kg. and thereafter remained negative. All four control animals died of acute trypanosomiasis within 14 days after the other groups had been given the drug. The blood of the 621C47-treated cattle was examined for trypanosomes on 71 occasions over a period of 100 days.

At the end of the experiment, 20 ml. of blood was taken from each calf of the group treated with 1.0 mg./kg. of 621C47, pooled and sub-inoculated into two clean calves. These animals remained free from trypanosomes.

TABLE II

EFFECT OF 621C47 AND 150C47 UPON DRUG-RESISTANT STRAINS OF *T. CONGOLENSE*

Drug	Dose mg./kg.	Cattle Infected with Dimidium-resistant *T. congolense*		Cattle Infected with Antrycide-resistant *T. congolense*	
		Ratio of Animals Cured to Animals Infected	Period Observed (Months)	Ratio of Animals Cured to Animals Infected	Period Observed (Months)
621C47	1·0	0/3	1·5	—	—
	2·0	2/4	1·5	0/6	2
	3·0	—	—	0/6	2
Antrycide dimethyl sulphate	5·0	4/4	3·8	0/3	2
Dimidium	1·5	1/3	1·5	—	—
—	Controls	0/3	2	0/3	2
150C47	1·0	—	—	0/6	2·8
	2·0	—	—	0/6	2·8
	3·0	—	—	5/6	2·8
Dimidium	1·5	—	—	0/3	2·8
—	Controls	—	—	0/5	2·8

Table II gives the results of treatment of cattle infected with drug-resistant strains of *T. congolense*.

They show that against an antrycide-resistant strain of *T. congolense*, 150C47 was active only at a very high dose-level, while 621C47 was inactive. The calves began to relapse 11 days after treatment with 150C47, and 14 days after treatment with 621C47. On the 71st day after treatment, the surviving calves from the groups given 621C47 were re-treated with 5.0 mg./kg. of antrycide methylsulphate and 2.0 mg./kg. of 621C47 simultaneously. The blood of all six animals remained negative (examined on 36 occasions over a period of 60 days), in contrast to the control animal which was positive. One calf of the re-treated group died from unknown causes.

621C47 had very little therapeutic effect upon *T. congolense* made resistant to dimidium. However, because of the chemical similarity of these two drugs, this result was not unexpected. An interesting observation in this experiment was that the calves treated with antrycide were apparently cured (blood examined on 59 occasions over a period of 111 days).

Experiments at Malakal

Therapeutic Experiments.—Twenty-four of the 29 calves infected with *T. congolense* were dosed with 2.0 mg./kg. of 621C47, with the remaining 5 left as controls. Similarly, 15 of the 19 calves infected with *T. vivax* were given 2.0 mg./kg. of 621C47, leaving 4 infected animals as controls.

After treatment a drop of blood from each calf was examined microscopically for trypanosomes on three successive days each week for 12 weeks. All the treated cattle were negative throughout this period. The controls remained infected, and three from each control group subsequently died from acute trypanosomiasis.

Two calves of the treated *T. congolense* group died, one from pleuropneumonia and the other from an unknown cause. A third calf had to be slaughtered on account of a broken leg. In the *T. vivax* group of calves, two died of pleuropneumonia.

Prophylactic Experiments. — Calves challenged with *T. congolense* one and two weeks after dosing with 2 mg./kg. of 621C47 remained negative throughout the duration of the experiment, whereas all calves challenged three, four and five weeks respectively after dosing became infected. All ten control calves were positive.

All animals challenged with *T. vivax* one week after dosing remained negative, but two of the five calves challenged two weeks after dosing developed infections of *T. vivax*. All calves challenged three and four weeks after dosing remained negative up to the end of the experiment. Nine of the ten control animals were positive.

It is unfortunate that the results with *T. vivax* were not as uniform as those with *T. congolense*. However, *T. vivax* is notorious for its irregular behaviour.

These experiments show that 2 mg./kg. of 621C47 will protect cattle from infection with *T. congolense* for only two weeks, and with *T. vivax* for one week. This agrees with the previous experiments which showed that *T. vivax* is more resistant to treatment with 621C47 than is *T. congolense*.

Toxicity of 621C47

In general, the toxic effects observed with 621C47 were similar to those reported for other phenanthridinium compounds. No calves died as a result of treatment with 621C47, but a delayed toxic effect, resembling photosensitization, was observed in one animal. This animal (Khartoum experiments) was originally infected with *T. congolense*, and the skin lesions were first noticed 57 days after subcutaneous injection with 4.0 mg./kg. of 621C47. The bull was white, with brown patches, and the lesions were found in the unpigmented areas at the base of the tail, the insides of the thighs, the back, and down the sides. The condition was typical of cases encountered in the Sudan on previous occasions, and took six months to clear. While it lasted the animal was irritable and lost condition.

Samples of serum and urine taken 91 days after the injection of the drug were analysed by Professor C. Rimington. No phylloerythrin was detected, although at this time the lesions were still severe.

At the site of injection some reaction was always present. After a dose of 2 mg./kg. swelling occurred within 3 days, sometimes becoming oedematous. After hardening, the swelling gradually disappeared, until after about 6 weeks only a slight thickening of the skin was found. The skin covering the swelling often broke down and a scar developed. Thus, of 16 calves treated with 2 mg./kg. of 621C47, 10 of them developed scars. This account describes the local reaction to the drug as seen in the Khartoum experiments when the cattle were examined weekly. The animals at Malakal were examined less frequently, but it was obvious that the reaction was much more severe here than at Khartoum.

This was probably because the Malakal cattle had to forage for food, thus dispersing the phenanthridinium solution in the subcutaneous spaces and aggravating the lesion mechanically. When the Malakal calves were examined 3 weeks after injection of the drug, every animal had a hard swelling, often measuring as much as 14 × 16 cm., and scars were observed in 56 of 104 calves. After 10 weeks a scar was found at the site of injection in almost every animal.

Two animals were given an intramuscular injection of 2.0 mg./kg. of 621C47 into the buttock and no swelling or scar developed with this route of injection.

As the toxicity of phenanthridinium compounds is revealed by the pathological picture of the liver (Goodwin and Chandler, 1952; Burdin, Plowright and Purchase, 1952), punch liver biopsies were done in animals treated with 621C47. Lack of time made it impossible to obtain a complete series from the same animals, but biopsies were made in various animals at different times after injection of the drug. Thus liver specimens were obtained from one calf 105 days after treatment with 1 mg./kg; from 4 calves 29 days after 2 mg./kg.; from 3 calves 47 days after 2 mg./kg. and 140 days after 2 mg./kg. Fat was observed in stained sections from one animal, and was present in cells lining the portal tracts. This animal had been treated with 2 mg./kg. of 621C47 and the biopsy specimen was taken 47 days after dosing. The other specimens were normal.

It was found that 150C47 gave a more severe local reaction than did 621C47; but no further observations on the effect of this drug were made.

Summary

1. Two phenanthridinium derivatives, 621C47 (2 : 7-diamino-9-α-thienyl-10-methylphenanthridinium chloride) and 150C47 (2 : 7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride), were given trials in the Sudan against *Trypanosoma congolense* and *T. vivax* in cattle.

2. Under laboratory conditions, a dose of 1 mg./kg. of 621C47, or of 150C47, eradicated infections of *T. congolense* in cattle; but *T. vivax* infections required 2 mg./kg.

3. Neither 621C47 nor 150C47 proved effective in non-toxic doses against an antrycide-resistant strain of *T. congolense*; nor was 621C47 effective against a dimidium-resistant strain of *T. congolense*.

4. Under field conditions in the S. Sudan, 2 mg./kg. of 621C47 eradicated *T. congolense* and *T. vivax* infections in calves.

5. 621C47 showed no prophylactic activity.

6. When given subcutaneously, 621C47 caused swelling, ulceration and scarring of the skin. A delayed toxic effect was seen in one animal after a large subcutaneous dose (4 mg./kg.). In two experiments, intramuscular injection gave no local reaction.

We would like to thank Mr. J. T. R. Evans, Director, Sudan Veterinary Service, for his help and advice throughout this work, Dr. L. P. Walls and Dr. T. Dewing, who prepared the phenanthridinium compounds, and Dr. L. G. Goodwin, who cut and prepared frozen sections of the liver biopsies. We are also grateful to Saad Mehanna, Dip. Vet. Sci. (Khartoum), for supervising the experiments at Malakal, and to Ibrahim Abdulla, head stockman at Khartoum North.

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