Field Trials with 3-Chloro-9-(4-Diethylamino-1-Methylbutylamino) Acridine, 10-Oxide Dihydrochloride (CI-423) as an Antimalarial Compound

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A new acridine derivative known as CI-423 has been developed by Parke-Davis and Company and has been extensively studied in the laboratory. Laboratory and clinical data have shown low toxicity in both animals and humans, as well as pronounced antimalarial activity in animals.[1, 2] A field trial in Liberia in 50 Africans showed considerable activity against *Plasmodium falciparum* infections.[3] Recently a field trial has been completed in Southern Rhodesia, where the drug was compared with Amodiaquine in a double blind study of 170 patients treated for *P. falciparum* or *P. malariae* infections with one of the two drugs as a single dose at various dosage levels; 140 returned for adequate follow-up and are reported.

Object of Trial

The trial was conducted in order to compare single doses of an entirely new antimalarial

with a known drug. A W.H.O. publication [4] states that among the 4-amino-quinolines, Amodiaquine (Camoquin) and Chloroquine are equally effective. This trial compared equal doses of CI-423 and a known standard—in this case Amodiaquine for efficacy.

Another grouping was set up to compare one-half the recommended dosage of Amodiaquine to one-third the recommended dosage of CI-423.* The actual amount of drug administered and a summary of the results are shown in Table II.

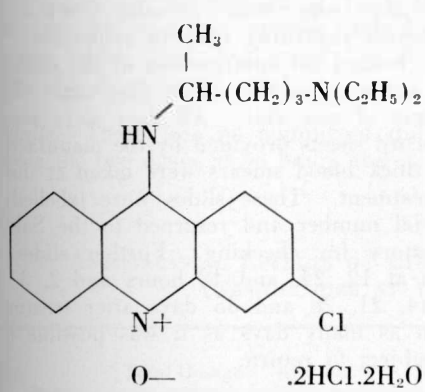
MATERIALS AND METHODS

Drugs

(a) *CI-423.*—CI-423 is a bitter-tasting, water-soluble, yellow crystalline substance which contains 84.1 per cent. active base; it is identified chemically by the following structure and name:

$C_{22}H_{28}ClN_3O.2HCl.2H_2O$ (494.88)

CN-22.241-2$_b$ -1$_b$ 3-chloro-9-(4-diethylamino-1-methylbutylamino)-*acridine*, 10-oxide, dihydrochloride, dihydrate



(b) *Amodiaquine* (*Camoquin*).—This was supplied as the dihydrochloride dihydrate (76.6 per cent. base); it is a water-soluble, orange-yellow crystalline powder.

Both drugs were formulated as finely divided powders in opaque gelatin capsules of appropriate size. The dosage schedule is shown in Table II.

Double Blind Trial

In order to obtain objective data the trial was conducted under double blind conditions. The drugs were contained in glass vials according to a random code series. The vials were code numbered in a random fashion and the code number was used in all subsequent identification of the subject during and after treatment; the code was not broken until the trial was completed and results correlated.

In each age group the same number of capsules were administered regardless of dosage; some capsules were filled with placebo in the case of the lower dosage levels.

The capsules were administered with water and one of us (E.T.R., F.B.R.) checked that they were, in fact, swallowed. Since children under three years of age were unable to swallow the capsules, we decided to open the capsules and mix the contents with sugar. This mixture was swallowed with little difficulty. As the treatment was completed six months prior to analysis of the results, and as no notes were made concerning the contents of the capsules, this procedure did not interfere with the double blind status of the trial, since it was impossible to recollect any individual treatment.

Area

This trial was carried out in Southern Rhodesia near the edge of the extensive old flood plain of the Zambesi river. The area is flat, heavily wooded, waterless for much of the year and very hot. The altitude is approximately 600 metres and the mean winter temperature is 20° C., while the summer mean is 30° C.; the trial was conducted during the winter months.

Rainfall is seasonal, occurring between November and May; the rest of the year is dry. During the rainy season surface water is abundant, the rivers flow and the malaria vector *Anopheles gambiae* breeds everywhere; malaria transmission is extensive. Once the rains cease the rivers rapidly dry up, and the only available water in the area is from boreholes or from holes dug in the river beds to water the stock. Malaria mosquitoes can breed in these small pools, and although malaria transmission has not been proved to occur during the winter months, it has not been disproved. This fact must be considered when the results are analysed.

Malaria Incidence

Malaria is hyperendemic; overall parasite rates vary between 55 per cent. and 70 per cent.,

*This somewhat unusual combination was chosen by the manufacturers in order to obtain data on lowest possible dosage levels.

with 100 per cent. incidence in children under five years of age. The majority of infections are due to *P. falciparum*, while *P. malariae* accounts for about 5 per cent. to 10 per cent. and *P. vivax* less than 1 per cent. The gametocyte ratio in the persons infected with *P. falciparum* averaged 17.5 per cent.; in adults it was 18 per cent. and in children between 9 per cent. and 20.5 per cent.

Nutritional State of Subjects

(W. Carr, Food Technologist)

One hundred and forty-six Africans of both sexes and all ages were included in the clinical nutrition survey. The largest percentage of subjects were children. Because of the difficulties of establishing exact ages, the weights of the subjects were divided into eight groups. All individuals in age groups under 16 years were underweight for height, but the adults were within normal ranges for weight. Clinical examination showed a very high incidence of goitre, numerous persons with dry, dull, horny skins and a small number of cases of follicular keratosis. The incidence of gynaecomastia in boys in their teens was high. No frank signs of deficiency disease, except goitre, were seen. The overall picture suggests that the children, at any rate, were receiving a diet deficient in vitamin A, iodine, calories and protein.

Selection of Subjects

Because of local conditions it was not possible to select only persons with clinical malaria for trial. In any case, with the high degree of immunity prevailing, clinical malaria is often obscured, particularly in the winter months, by influenza, bronchitis and other diseases. In view of this, selection was made on the basis of whether or not parasites could be demonstrated in a single thick blood film. In order to ensure that the persons with the highest degree of parasitaemia were selected, only those with parasites showing in the first five fields on a slide on the day prior to beginning the trial were chosen. The mean age, weights and parasite densities of subjects are shown in Table I.

The subjects selected were offered liberal amounts of sweets, sugar, salt or maize meal whenever they came to have a blood slide made. This materially assisted in obtaining co-operation, particularly that of the children, many of whom had to travel six or seven miles every day to the clinic. Originally 250 out of 890 persons were selected as suitable for treatment, but finally 170 were treated, of whom only 140 returned on a sufficient number of occasions to provide data for analysis.

Method of Assessment

Subjects who were parasite positive were questioned regarding malaria symptoms. They were checked for enlargement of the spleen and each subject was treated at that time with the contents of one vial. All data were recorded on record sheets provided by the manufacturers. Two thick blood smears were taken at the time of treatment. These slides were labelled with the vial number and returned to the Salisbury laboratory for checking. Further slides were taken at 12, 24, and 48 hours and 2, 3, 4, 6, 10, 14, 21, 28 and 35 days after treatment—or on as many days as it was possible to get the subject to return.

Table I

Average Ages, Weights and Density of Parasitaemia in Persons Selected for Treatment

Age Groups	Number	Average Age	Average Weight (Kg.)	Parasite Counts		
				Number	Average Parasite Count/100 Leucocytes	Number with Less than 10 Parasites/100 Fields
Adults	30	23	52	16	45	14
6-12 years	80	8	27	66	60	14
2-5 years	37	4	14	35	88	2

On each return visit the subjects were asked whether they had been ill. Evidence for enlargement of the spleen was sought on two occasions during the five weeks.

Slide Examination

Blood slides were stained with Giemsa stain and examined by one of us (E.T.R., R.F.); 100 oil-immersion (x100) fields were examined on each slide. Comparison counts between the two observers indicated less than 10 per cent. error between the counts. Therefore slides were examined at random by the two observers; cross checks were made occasionally or if untoward results were observed. Counts were made against 100 leucocytes if parasites were reasonably dense or in 100 fields if sparse. (There were approximately 25 leucocytes on an average thick film field.) Mean pre-treatment counts are shown in Table I.

Results

Trophozoite Clearance at 600 mg. Dosage Rate

The results are summarised in Table II. As there was no apparent species difference in clearance rates, they are given for *P. falciparum* and *P. malariae* combined.

Adults.—There was no significant difference between the two drugs at 48 hours and 72 hours after treatment, and although slightly higher cure rate with Amodiaquine is apparent at 21 days, this is not significantly different; what is interesting, however, is that at 21 days neither drug had given a 100 per cent. clearance rate.

6-13 Year Age Group.—There are no significant differences in the clearance rates between the drugs; again 100 per cent. cure rate was not achieved with the proportionate for age dose level.

2-5 Year Age Group.—There is a higher clearance rate with CI-423 up to 72 hours, but at 21 days the advantage is very significantly with Amodiaquine.

Trophozoite Clearance at 300 mg. Amodiaquine or 200 mg. CI-423

Adults.—There is a preference for Amodiaquine over CI-423 at 48, 72 hours and 21 days.

6-13 Year Age Group.—There is a significantly higher cure rate with Amodiaquine than with CI-423, which is particularly marked at 21 days.

2-5 Year Age Group.—Amodiaquine is again significantly more effective than CI-423 at 72 hours and 21 days. Neither drug is, however, particularly effective at these low dose levels.

Table II

Antimalarial Effects of CI-423 and Amodiaquine, as Reflected by Blood Examinations at Various Intervals After Treatment

Drug	Total Dosage mg. in Single Dose	Age of Patients in Years	No Patients Studied	48 Hours		72 Hours		21 Days	
				Number Patients	% Neg.	Number Patients	% Neg.	Number Patients	% Neg.
CI-423	600	> 13	8	8	100	4	100	5	80
Amodiaquine	600	> 13	9	7	86	6	100	8	88
CI-423	300	6-13	18	16	56	15	80	16	94
Amodiaquine	300	6-13	19	18	67	16	75	17	94
CI-423	150	2- 5	7	7	85	2	100	4	25
Amodiaquine	150	2- 5	12	11	55	4	75	10	90
CI-423	200	> 13	8	7	71	8	75	5	80
Amodiaquine	300	> 13	5	5	80	5	100	3	100
CI-423	100	6-13	18	18	56	15	67	15	47
Amodiaquine	150	6-13	19	17	71	14	93	18	89
CI-423	50	2- 5	8	8	38	6	17	7	0
Amodiaquine	75	2- 5	9	8	38	6	67	9	56

If one compares all subject groups, there is no statistically significant difference at the 48-hour and 72-hour levels, but at 21 days the Amodiaquine group showed 86 per cent. negatives while 60 per cent. negatives were seen in the CI-423 group, and chi-square test indicates a less than 0.005 chance that the two treatments were made in populations with the same proportion of negative and positive subjects. If, however, one considers the two drugs at the two different dosage rates, it then becomes apparent that (1) this difference is due to the lower dosage used with CI-423; (2) the differences are a function of dosage; (3) at the same dosage levels the two drugs are equally effective.

The difference is particularly marked in the youngest age groups who had the lowest actual dosage. The relatively low toxicity of CI-423 also suggests that at a higher dosage rate for children it would prove advantageous in achieving a cure in a single treatment.

Effect of Drugs at Low Dosages

Clyde [5] found in East Africa that he could obtain a cure with dosages lower than the advised 600 mg. Chloroquine base. In the trial outlined above it was found that with lower dosage levels the cure rate fell off considerably (Table II). This result is probably due to a difference in the immunity status of the subjects.

Rapidity of Cure

The data shown in Table II suggest that there is no significant difference in the rate of reduction of parasitaemia between the two drugs. Detailed examination of the original figures suggests that the clearance rate depends to some extent on the original intensity of parasitaemia. However, without detailed four-hourly or eight-hourly counts during the first 72 hours we consider that it would be imprudent to make any definite statement on the differences in rate of reduction of parasitaemia between the two drugs.

Further trials may clarify this point.

Gametocyte Clearance

No difference was noted between the two drugs in their effects on gametocytes, namely, that they showed no gametocytocidal activity.

Toxicity

No toxic side effects were noted during the trial for either drug. (Parke, Davis clinical data [1] suggests that oral toxicity of CI-423 is very low and that at therapeutic doses toxic side effects are unlikely to occur.)

SUMMARY

The new acridine drug CI-423 was compared with Amodiaquine in Southern Rhodesia for treatment of malaria in a semi-immune population. The drug was given in two dosage schedules; the first was a single dose of 600 mg. Amodiaquine, or 600 mg. of CI-423 to adults, with proportionately smaller doses for children. In the second series patients received a 50 per cent. dose of Amodiaquine or a 33 per cent. dose of CI-423.

At the end of 21 days there was no significant difference between the drugs at the full dosage level, but Amodiaquine was significantly better at the lower levels. It seems probable that this difference was due to the inequality of dosages.

No difference in effect on gametocytes could be found between the two drugs.

No toxic effects or intolerance were noted. The new drug, CI-423, appears to hold promise as an antimalarial, particularly in single dose therapy, since its efficacy at similar dosage is comparable with Amodiaquine.

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