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EFFECTIVENESS OF SINGLE DOSE TREATMENT WITH CHLOROQUINE OF MALARIA IN WEST AFRICA AND MEASUREMENT OF CHLOROQUINE URINARY EXCRETION

by

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Summary — A study on the *in vivo* sensitivity of *Plasmodium falciparum* to chloroquine was carried out among 7 to 14 year old schoolchildren in a rural area near Bobo-Dioulasso (Burkina Faso). A single oral dose of chloroquine of 10 mg/kg induced a complete disapppearance of all asexual stages from peripheral blood lasting two weeks. Measurement of urinary chloroquine excretion by a colorimetric method did not enable to distinguish the further spontaneous consumers of chloroquine.

KEYWORDS: *Plasmodium falciparum*; Chloroquine Sensitivity; Chloroquine urinary Excretion; Malaria; West Africa.

Chloroquine remains the main drug used by the West African population in the treatment of malaria. In order to establish *Plasmodium falciparum* sensitivity to this drug, we made an *in vivo* test using a single 10 mg/kg dose of chloroquine *per os*.

Parasitological rates were measured during five weeks. Urinary excretion of chloroquine and/or its metabolites was measured during the last three weeks in order to identify the individuals who had further spontaneous chloroquine intakes.

1. Material and methods

The study was carried out among pupils attending primary school at Loroforousso, a village located fifteen kilometers from Bobo-Dioulasso (Burkina Faso), in a savanna area with a dry season from November to May and a rainy season from June to October. Yearly average rainfall is 1000 mm. Malaria transmission is seasonal and intense: unprotected individuals receive on the whole about 150 infected bites each from June to November (5).

Loroforousso had no dispensary nor effective primary health agent at the time of this study. Drugs were available in the city of Bobo-Dioulasso, while health care could be given in the neighbouring villages dispensaries.

151 children, 7 to 14 year old, were used for this survey. Parasitological prevalence and density and *Plasmodium* species determination were measured both on thick and thin smears for each individual. Detection threshold of parasitaemia was estimated by the observation of about 100,000 RBC at 100

parasitized red blood cells by mm^3 of blood (PRBC/mm^3). Urinary chloroquine excretion was evaluated by the semi-quantitative colorimetric method described by Bergqvist *et al.* (4). This method involved an ion-pair extraction with dichloromethane using the acid-base indicator bromothymol blue as counter-ion.

Urine samples were collected in the morning at school without difficulties, though it was not the first miction of the day, and were treated on the same afternoon. Chloroquine extraction by bromothymol blue was made only once, while Bergqvist made it twice. As no spectrophotometer was available we could not have a great precision in the quantification and we did not see any advantage in making the extraction twice. Colorimetric reaction was optically measured by two observers in comparison with a standard scale of 0, 25, 50, 100 and 200 micromoles of chloroquine per liter.

Results were analysed using parametric tests (comparison of two means, test of correlation).

2. Survey calendar

The survey started on the 10th of June 1986 (DO) and ended on the 15th of July, in between malaria transmission and non-transmission season.

Weights of healthy children (i.e. apyretic, having no complaint of distress or disease) were measured. These children received chloroquine (Nivaquine® Specia, tablets of 100 mg) at a 10 mg/kg posology. The drug was swallowed under our control. Peripheral blood was taken in order to realize thick and thin smears.

At D+7, blood smears were performed.

At D+14, D+17, D+21, D+24, D+28, D+31, D+35, both blood smears and collection of urine were done.

3. Results

3.1. *Parasitological data*

66% of the 7 to 10 year old children and 19% of the 11 to 14 year old children had asexual stages of *Plasmodium* in their blood at DO. Arithmetical average parasitic density of the positives was higher in the younger (2280 versus 689 PRBC/mm^3, border-line statistical significance, $p < 0{,}10$). *P. falciparum* was observed alone in 81% of the infections. *P. malariae* was present in the remaining associated with *P. falciparum.* The *P. falciparum* gametocytic index was 20% (tables 1 and 2).

The single use of chloroquine was very effective against asexual stages:

At D+7, only one child had few trophozoites of *P. falciparum* and at D+14, none had any trophozoites in peripheral blood.

At D+17, *P. falciparum* trophozoites were found in 10 children (7%).

TABLE 1

Parasitological data from 7 to 10 year old children in June and July 1986, village of Loroforousso (Burkina Faso)

	DO	D+7	D+14	D+17	D+21	D+24	D+28	D+31	D+35
Plasmodium asexual stage prevalence	69/104 = 66 %	1/104 = 1 %	0/103 = 0 %	7/104 = 7 %	19/104 = 18 %	30/103 = 29 %	50/104 = 48 %	59/104 = 57 %	58/104 = 56 %
Species repartition	Pf = 52 Pm = 8 Pf+Pm = 9	Pf only	—	Pf only	Pf only	Pf only	Pf only	Pf = 58 Po = 1	Pf = 57 Po = 1
P. falciparum gametocyte prevalence	16/104 = 15 %	25/104 = 24 %	14/103 = 14 %	10/104 = 10 %	6/104 = 6 %	1/103 = 1 %	1/104 = 1 %	4/104 = 4 %	0/104 = 0 %
Parasitic density in PRBC/mm^3	2280 SD:2938	400 SD:0	—	971 SD:1391	9305 SD:27985	3500 SD:9535	5648 SD:13712	6719 SD:20426	5331 SD:13414

TABLE 2

Parasitological data from 11 to 14 year old children in June and July 1986, village of Loroforousso (Burkina Faso)

	DO	D+7	D+14	D+17	D+21	D+24	D+28	D+31	D+35
Plasmodium asexual stage prevalence	9/47 = 19 %	0/47 = 0 %	0/47 = 0 %	3/46 = 6 %	2/47 = 4 %	8/47 = 17 %	11/47 = 23 %	15/47 = 32 %	26/47 = 55 %
Species repartition	Pf = 4 Pm = 3 Pf+Pm = 2	—	—	Pf only	Pf only	Pf only	Pf only	Pf only	Pf only
P. falciparum gametocyte prevalence	14/47 = 30 %	14/47 = 30 %	10/47 = 21 %	6/46 = 13 %	2/47 = 4 %	2/47 = 4 %	3/47 = 6 %	1/47 = 2 %	2/47 = 4 %
Parasitic density in PRBC/mm^3	689 SD:975	—	—	100 SD:0	100 SD:0	388 SD:390	300 SD:240	1573 SD:3569	1942 SD:4369

In the tables 1 and 2, the parasitic densities have been computed using the arithmetical mean from the positive children.

Pf: *P. falciparum* Pm: *P. malariae* Po: *P. ovale*

At D+21, trophozoites were found in 21 children (14%).

At D+31, trophozoites prevalence was equivalent to the prevalence found at DO (49%).

Gametocytic index dropped from 20% at DO to 1.5% at D+35.

We did not observe reappearence of *P. malariae* while *P. ovale* was observed in two children at D+31 and D+35.

3.2. *Detection of chloroquine in the urine*

7 to 10 year old and 11 to 14 year old children showed similar urinary excretion levels of chloroquine and/or its metabolites.

At D+14, all the children had chloroquine in their urine. The mean excretion level was 85 micromoles/liter. Further on we observed a decrease in the excretion. At D+35, 13 children had no detectable chloroquine in urine and the mean excretion level was 55 micromoles/liter. The difference between D+14 and D+35 was statistically highly significant ($\varepsilon = 5.28$ $p < 0.001$), however the decrease was not uniform (table 3).

On the other hand, during the three last sampling sessions, we observed 16 children showing a simutaneous increase in their urinary excretions of chloroquine and in their parasitaemias.

TABLE 3

Urinary chloroquine excretion of 7 to 14 year old children in June and July 1986, village of Loroforousso (Burkina Faso). The excretion in measured by the semi-quantitative colorimetric Bergqvist's test.

	D+14	D+17	D+21	D+24	D+28	D+31	D+35
n	148	148	150	151	148	148	141
Arithmetical mean	85.5	37.5	62.0	74.5	45.0	58.0	55.0
SD	48.1	27.1	48.4	61.2	40.8	53.2	48.1

Chloroquine and/or its metabolites are expressed in micromoles/liter.

4. Discussion and conclusion

This field study shows the effectiveness in the year 1986 of a single dose of 10 mg/kg chloroquine against *P. falciparum* in semi-immune children living in a rural area of West Burkina Faso. It confirms a previous study in the same area (2), though an *in vitro* resistant *P. falciparum* strain has already been observed in Burkina Faso (3).

Single dose of chloroquine induced the disappearance of asexual stages in peripheral blood during two or three weeks, and probably a fall in the gametocytic stages after a longer time.

The first examination of blood at DO showed a 52% prevalence of *Plasmodium*, which was very high for the season. It proves that the children of the village were not regular consumers of antimalarial drugs.

Determination of chloroquine urinary excretion was done by Bergqvist's method in the purpose of identifying which children had had further chloroquine intakes during the survey.

The observation of 16 children with simultaneous important increase of their parasitaemias and chloroquine excretions (50 micromoles/liter and more) was surprising since chloroquine seemed to be highly effective between DO and D14.

Three explanations seem possible to the increase of chloroquine urinary excretion:

— Imprecision in the measurement of chloroquine in urine, mainly due to the variations in daily urinary volumes and in the individual urinary chloroquine excretion (1, 6).

— Cross reaction of the colorimetric method between chloroquine and other molecules, drugs or else.

— Further intakes of chloroquine, which are not consistent with the increase of parasitaemias.

In conclusion, we observed the effectiveness of a single oral dose of 10 mg/kg of chloroquine against *P. falciparum.* The semi-quantitative urinary test appeared to be lacking of precision in determining the spontaneous chloroquine consumers.

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Efficacité d'une dose unique de chloroquine dans le traitement du paludisme en Afrique de l'Ouest. Mesure de l'excrétion urinaire de la chloroquine.

Résumé — Dans un village proche de Bobo-Dioulasso, en juin 1986, en début de saison de transmission des paludismes, parmi 151 enfants âgés de 7 à 14 ans, 78 soit 52 % sont porteurs d'hématozoaires. Une prise unique de chloroquine *per os* à la dose de 10 mg/kg entraîne une disparition complète des stades asexués pendant deux semaines. Des trophozoïtes de *Plasmodium falciparum* apparaissent à partir de J + 17. A J + 31, la prévalence parasitaire est équivalente à celle de JO. La mesure de l'excrétion urinaire de la chloroquine et/ou de ses métabolites est faite par la méthode de Bergqvist simplifiée : la comparaison des réactions colorimétriques avec celles d'une gamme étalon est effectuée à l'œil et non avec un spectrophotomètre. Cette technique n'a pas permis de mettre en évidence les consommateurs spontanés de chloroquine. Les raisons de cet échec sont discutées.

Doeltreffendheid van éénmalige malaria behandeling met chloroquine in West Afrika en meting van de chloroquine excretie in de urine.

Samenvatting — De gevoeligheid van *Plasmodium falciparum* voor chloroquine werd *in vivo* bestudeerd bij schoolkinderen tussen 7 en 14 jaar oud, in een rurale streek bij Bobo-Dioulasso (Burkina Faso). Een éénmalige dosis chloroquine (10 mg/kg) deed alle asexuele vormen volledig uit het perifeer bloed gedurende twee weken verdwijnen. Chloroquine excretie in de urine, gebruik makend van een colorimetrische methode, liet niet toe diegenen die spontaan verder chloroquine gebruikten, op te sporen.

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REFERENCES

1. Ba BB, Estadieu M, Gouezo F, Durand A, Viala R, Delmont J, Ranque Ph : Suivi thérapeutique par la détermination de chloroquine dans l'urine après fixation sur papier échangeur d'ions. Document non publié WHO/MAL/86.1026.

2. Baudon D, Roux J, Benthein F, Carnevale P, Molez JF : Etude de la sensibilité de *Plasmodium falciparum* à la chloroquine par les épreuves *in vivo* dans une zone de savane arbustive

de Haute-Volta à paludisme hyperendémique. Document non publié WHO/MAL/84.1011.

3. Baudon D, Devoucoux R, Roux J, Sondo B: Etude de la sensibilité de *Plasmodium falciparum* à la chloroquine dans une zone de savane du Burkina Faso. Bull. Soc. Path. Exot., 1984, **77**: 658-665.
4. Bergqvist Y, Hed C, Funding L, Suther A: Determination of chloroquine and its metabolites in urine: a field method based on ion-pair extraction. Bull. WHO, 1985, **63**: 893-898.
5. Robert V, Gazin P, Ouédraogo V, Carnevale P: La transmission du paludisme en zone de savane arborée et en zone rizicole des environs de Bobo-Dioulasso. Ann. Soc. belge Méd. Trop., 1985, **65**, Suppl. 2: 201-214.
6. Verdier F, Pussard E, Blayo MC: Pharmacocinétique des antipaludéens: quinine et méfloquine, halofantrine, quinghaosu, amino-4-quinoléines. Méd. Trop., 1986, **46**: 329-343.