DOC. TEC. OCEAC N°685/SEM/LAB May 1989

IN VIVO MONITORING OF CHLOROQUINE SENSITIVITY OF *PLASMODIUM FALCIPARUM* IN EDEA, SANAGA MARITIME DISTRICT, SOUTH WEST CAMEROON

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Summary

The town of Edea is located in South West Cameroon in a zone of chloroquine resistance. Falciparum malaria is hyperendemic. We have performed an *in vivo* study of chloroquine sensitivity in 190 school children 6 to 12 years of age. In three days they are given 25 mg/kg chloroquine orally. Parasite densities are controlled on D3 and D7.

123 children (64.5%) had positive blood films for malaria at D0. The splenic index was 27%.

68 children met the enrollment criteria. Their GMPD was 1585 PRBC/mm^3. On D3, 16 children (23.5%) still carried parasites with a GMPD of 40, while on D7 12 children (17.5%) remained parasited with a GMPD of 141 PRBC/mm^3. Two of them had a relatively high parasitaemia. We conclude that chloroquine remains the drug to be used at first intent.

Résumé

ETUDE *IN VIVO* DE LA SENSIBILITE A LA CHLOROQUINE DE *PLASMODIUM FALCIPARUM* A EDEA, DEPARTEMENT DE LA SANAGA MARITIME, SUD-OUEST DU CAMEROUN

La ville d'Edea est située au sud-ouest du Cameroun dans une zone où la chloroquinorésistance est connue depuis 1985. Le paludisme y est hyperendémique. En mai 1989, nous avons réalisé un test de sensibilité *in vivo* chez 190 écoliers âgés de 6 à 12 ans. Ils reçoivent 25 mg/kg de chloroquine *per os* en 3 jours. Les densités parasitaires sont contrôlées à J3 et J7 sur goutte épaisse.

123 enfants (64,5%) sont porteurs de parasites à J0. L'indice splénique est de 27%. 68 enfants sont inclus dans l'étude. Leur parasitémie moyenne initiale est de 1585 GRP/mm^3. A J3, 16 d'entre eux (23,5%) sont porteurs d'hématozoaires à une densité moyenne de 40 GRP/mm^3. A J7, 12 (17,5%) ont encore des parasites, à une densité de 141 GRP/mm^3. Deux individus ont une parasitémie relativement élévée. La chloroquine reste l'antimalarique à utiliser en première intention dans cette région.

Introduction

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World areas with chloroquine resistant falciparum malaria are progressively spreading in Africa from East to West. In the South West of Cameroon chloroquine resistance is known since1985.(12)

Since then various studies have confirmed this situation (8) and assessed that chloroquine resistance is focussed on three main areas in Cameroon: the coast at Limbe (1), at Kribi (7) and in the city of Yaoundé.(4)

Edea is situated on the banks of the river Sanaga in a tropical rain forest zone. Edea is the chief town of the Sanaga Maritime district with one of the most important industrial centers of Cameroon, the aluminum factory ALUCAM. Malaria is considered to be hyperendemic in this area. (6)

Although situated in the center of the principal zone of chloroquine resistance Edea has never been the subject of a study to chloroquine sensitivity. Macaigne *et al* have studied amodiaquine in ALUCAM children and found good effectiviness.(9) We decided to carry out a study in Edea because of massive chloroquine administration, particularly (free of charge) under ALUCAM employees and their families and the fact that medical centers report an increasing number of consultations and hospitalizations for malaria since 1986.

Materials and methods

The quarter Pongo can be regarded as representative for the town of Edea. There are two primary schools of which the catholic Saint Martin school has 664 pupils divided over 11 classes. Four classes with about 200 pupils in the age range between 6 and 12 years have been studied.

In hyperendemic areas of Africa 30 days follow-up cannot be achieved without exposure to reinoculation by sporozoites. In May 1989 we carried out the simplified *in vivo* test over 7 days according to the method given by the OCEAC. (5)

All pupils present at the first day of the study (D0) were examined on spleen rate, weight and fever. We interrogated wether the children had access to the free medical service of ALUCAM. Blood was taken for thin and thick smears.

Detection of parasites was done on thick smear. The identification of *Plasmodium* species was done on thin smear. The parasite density was established by counting 30 fields (about 600 leucocytes) of the thick smear and/or by counting 100 fields (about 20.000 erythrocytes) on the thin blood film. The density was determined on the base of 8000 leucocytes and 4 million erythrocytes per mm^3 of blood volume.

All children were given 10 mg/kg of chloroquine *per os* on D0, the same dose on D1 and a dose of 5 mg/kg on D2 resulting in a total dose of 25 mg/kg in three days. Administration was done under supervision of intake until at least 15 minutes afterwards. Parasite density was controlled at D3 and D7 by thick smear.

Results

190 children were screened. Their mean age was 9 years with a mean weight of 26 kg. 5 children had fever on D0.

60 children had access to the ALUCAM medical service.

Parasitological findings

123 children (64.5%) had positive blood films for malaria. In 98% of the cases, *P. falciparum* was present. *P. malariae* was seen in 6.5% of the cases and *P. ovale* in 1%.

The geometric mean parasite density (GMPD) in subjects with parasites was 525 PRBC/mm^3 without age variation. The gametocyte index of *P. falciparum*, 3.5%, was low. The splenic index was 27%. There was a significant negative correlation between age and spleen size ($r = 0.16$, $p < 0.05$). Average enlarged spleen according to Hackett (3) was 1.64 (S.D. 0.72).

There is a positive correlation between spleen size and parasite density ($r = 0.19$, $p < 0.05$). There was a slight difference in spleen rate between the ALUCAM and non-ALUCAM group (Table 1).

Of the investigated children 1.5 % were carrier of microfilaires of *Mansonella perstans*. One child had microfilaires of *Loa loa*.

Effectiveness of chloroquine

The subjects allowed in the sensitivity test were those with a parasitaemia on D0 exclusively of *P. falciparum* with parasite counts of at least 500 trophozoites/mm^3 which have completed their 3 days treatment on D0, D1, D2, or D3. We didn't observe complaints of pruritis. Only one child was dropped from the study on D1 for excessive vomiting at the time of the treatment. 68 children met the enrollment criteria; their mean age, 9 years, and mean weight, 26 kg, being not different of the whole group. Their geometric mean parasite density (GMPD) was of 1585 PRBC/mm^3 .

On D3 16 children (23.5%) still carried parasites with a GMPD of 40, while on D7 12 children (17.5%) remained parasited with a GMPD of 141. These didn't differ from the whole group in age nor weight. Their initial parasite density was the same of those included in the test.

Two subjects, both from the non-ALUCAM group, showed a persistant parasitaemia (table 2).

Remarkably there is no parasitological difference found between the ALUCAM and non-ALUCAM children included in the test (table 3).

Discussion

The spleen rate (26%) differs very significantly from the one observed in a neighbouring rural community ($p < 0{,}001$).(2) The slight difference in the spleen rate between ALUCAM and non ALUCAM children indicates a trend that antimalarial drugs are most frequently being used in the first group. The high parasite rate is classic for Central Africa. (10,13) There are no remarkable age or quarter differences.

Chloroquine is used at a dose of 25 mg/kg *per os* in three days. The intake of tablets is supervised. Chloroquine blood and/or urine level are not controlled, thus it remains impossible to confirm that all children had a sufficiently high chloroquine blood level (> 65 nmol/l). (11)

In 68 children admitted to the study, 12 (17.5%) have demonstrated *in vivo* parasitological resistance mostly at a low level. Four of them have early RI resistance and seven a typical RII resistance.

In the case of the child who had fever on D0 and a parasitaemia of 10,000 PRBC/mm^3, we observe a lack of effectiveness of the chloroquine which requires an alternative treatment.

Conclusion

The *in vivo* 7 days test performed in a small town in Southwest Cameroon shows that in May 1989 chloroquine is still effective in a great majority of school children. Chloroquine remains the treatment of first intent in case of malaria in this area.

References

(1) Brasseur P.; Druilhe P.; Kouamouo J.; Brandicourt O.; Danis M.; Moyou S.R.:
High level of sensitivity to chloroquine of 72 *Plasmodium falciparum* isolates from southern Cameroon in January 1985.
Am. J. Trop. Med. Hyg., 1986, 35 (4): 711-716

(2) Gazin P.; Le Goff G.; Ambassa P.; Mulder L.; Carnevale P.:
[Study of malaria at Edea and Mbebe. First results].
Doc. Tec. OCEAC,1989, n° 680

(3) Hackett L.W.:
Spleen measurements in malaria.
J. Nat. Mal. Soc., 1944, 3: 397-403

(4) Hengy C.; Garrigue G.; Abisegue B.; Ghogomu N.; Gazin P.; Gelas H.; Kouka Bemba D.; Lebras J. et Jambou R.:
[Surveillance of chemosensitivity of *Plasmodium falciparum* in Yaoundé and environments - Cameroun. *In vivo* and *in vitro* study].
Bull. Soc. Path. Ex., in press

(5) Jambou R.; Gazin P.; Ghogomu N.; Mfundu D.; Trebucq A.; Hengy C.:
[Proposition of a protocol for a simplified *in vivo* test on 7 days].
Bull. OCEAC, in press

(6) Josse R.; Merlin M.; Combe A.; Josseran R.; Le Hesran J.; Avennec F.; Ngnintedem B.; Nkongtso A.M.; Kamwa M.; Eboumbou J.T.; Tribouley J.; Ripert C.:
[A comparative study of malaria indexes in Yaounde, Nanga Eboko and Edea (Cameroon)].
Méd. Trop., 1988, 48, 3: 201-208

(7) Kouamouo J.; Enyong P.; Brasseur P.; Moyou S.R.; Druilhe P.:
[New focus of chloroquine-resistant malaria in a forestry zone in Cameroon].
Bull. Soc. Path. Ex., 1987, 80 : 452-458

(8) Le Bras J.; Simon F.; Ramanamirija J.A.; Calmel M.B. *et al*:
[Sensitivity of *Plasmodium falciparum* to quinolines and therapeutic strategies: comparison of the situation in Africa and Madagascar between 1983 and 1986].
Bull. Soc. Path. Ex., 1987, 80: 477-489

(9) Macaigne F.; Combe A.; Eboumbou J.; Garnier T.; Michel R.; Ripert C.:
[*In vivo* sensitivity of *Plasmodium falciparum* to amodiaquine in the town of Edea (Cameroon)].
Bull. Soc. Path. Ex., in press

10) Richard A.; Lallemant M.; Trape J.F.; Carnevale P.; Mouchet J.:
[Malaria in the forest region of Mayombe, Republic of Congo. Parasitological observations].
Ann. Soc. Belge. Méd. Trop., 1988, 68: 305-316

11) Salako L.A.; Aderounmu A.F.; Walker O.:
Influence of route of administration on the pharmakinetics of chloroquine and desethylchloroquine.
Bull. WHO, 1987, 67: 47-50

12) Sansonetti P.J.; Le Bras J.; Verdier F.; Charmot G.; Lapresle C.:
Chloroquine resistant *Plasmodium falciparum* in Cameroon.
The Lancet, 1985, i: 1154-1155

13) Trape J.F.:
Malaria and urbanization in Central Africa: the example of Brazzaville. Parasitological and serological surveys in urban and surrounding rural areas.
Trans. Roy. Soc. Trop. Med. and Hyg., 1987, 81, suppl. 2: 26-33

[ ] Publication in French

Table 1: Malarial indices of Alucam and non-Alucam children in May 1989 before antimalarial treatment

	Alucam	Non Alucam
number (n=)	60	130
parasite rate	62%	66%
part of P. ovale or P. malariae in infections	3.3%	5.5%
GMPD *	447	501
spleen rate	22%	29%
AES **	1.46 (SD 0.66)	1.70 (SD 0.74)

Table 2: Characteristics of 2 children with low *in vivo* response of *Plasmodium falciparum* to chloroquine

Age	Sex	Weight	D0 (***)	D3	D7	remarks
7	F	19	750	110	1000	Gametes D3
8	F	20	10000	350	8000	Fever D1

Table 3: Prevalence and geometric mean parasite density of children included in the *in vivo* chloroquine test

		D0	D3	D7
Parasite rate	Alucam	n=21	24%	19%
	nonAlucam	n=49	22%	16%
GMPD (*)	Alucam	1445	26	76
	nonAlucam	1660	50	190

* Geometric Mean Parasite Density expressed in parasited red blood cells /mm^3 of blood volume

** Average Enlarged Spleen according to Hackett

*** Parasite density on day 0, day 3 and day 7