RESEARCH NOTE

In Vitro Responses *of Plasmodium falciparum* Isolates to Five Antimalaria Drugs in French Guiana during 1994 and 1995

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In vitro susceptibility of *Plasmodium falciparum* has been studied in French Guiana in the late 80's by JP Dedet et al. (1988 *Bull Soc Path Ex 81:* 88-93) and F Gay et al. (1992 XXII congrès SMAGF Cayenne 7-11 avril: 32-33). Since this period epidemiological changes occurred in the Maroni valley, region with the higher level of endemicity, because of the desorganization of the health centers on the Suriname side and the increase of goldminer settlements which are not well controlled on both sides. With this point of view and considering the evolution of the susceptibility of *P. falciparum* in French Guiana, *in vitro* test were carried out with five drugs commonly used in the treatment of malaria: chloroquine, amodiaquine, quinine, mefloquine and halofantrine.

Blood samples (10 ml) were collected in Sodium Heparinate tubes from patients who attended a medical center or a hospital in French Guiana and in whom the diagnosis of *P. falciparum* infection was microscopically established. Then blood samples were sent to our Institute in Cayenne. These patients included mild and severe cases, according to DA Warrell et al.'s case definitions (1990 *Trans R Soc Trop Med Hyg 84* (Suppl 2): 1-65).

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The *in vitro* tests were carried out according to RE Desjardins et al. (1979 *Antimicro Agents Chemother 16:* 710-718) technique, modified by P Brasseur et al. (1986 *Am J Trop Med Hyg 35:* 711-716). The test consists of a one step isotopic 48 hr microtest. Five antimalarial drugs were used: chloroquine diphosphate (Sigma, France), amodiaquine hydrochloride since March 1995 (Laboratoire Roussel, France), quinine hemisulfate (Sigma, France), mefloquine hydrochoride (Produits Roche, Suisse), halofantrine hydrochloride (Laboratoire SK&F, France). The final two fold dilutions in the wells of each drug (as base) ranged from 1,875 to 7.3 nM, 843 to 1.65 nM, 3,700 to 14.45 nM, 793 to 3.1 nM and 32 to 0.0625 nM, respectively. The 50% effective concentrations (EC_{50}) were calculated using log dose response probit analysis. Drug resistances of a *P. falciparum* isolate were established if EC_{50} >100 nM for chloroquine base, EC_{50} > 60 nM for amodiaquine base, EC_{50} > 500 nM for quinine base, EC_{50} > 30 nM for mefloquine base, EC_{50} > 6 nM for halofantrine base and drug susceptibility was established if EC_{50} < 80 nM for chloroquine base, EC_{50} < 40 nM for amodiaquine base, EC_{50} < 300 nM for quinine base, EC_{50} < 15 nM for mefloquine base, EC_{50} < 4 nM for halofantrine base (Brasseur et al. *loc. cit*., R Thor et al. 1994 *Bull Epid Hebdo 38:* 1-3).

Our Institut received 44 samples in 1994 and 36 samples in 1995. Twelve samples were discarded in 1994 and 9 in 1995 (too old samples, low parasitemia, mixed infestation with *P. vivax*). Therefore 32 and 26 tests were carried out in 1994 and 1995 respectively. Sixteen tests in 1994 and 5 in 1995 were not valid. The explanations are numerous: poor condition of the sample (too old, bad cold storage), antimalarial treatment before blood sampling, low parasitemia, etc.

The results of the 16 valid tests in 1994 and the 22 in 1995 showed that only one isolate was susceptible to chloroquine and 100% of the isolates were susceptible to mefloquine and halofantrine. 14% and 26% of the isolates were resistant and intermediate respectively to quinine in 1995, while all the isolates were susceptible in 1994. Seventeen of the 18 isolates tested were susceptible to amodiaquine. Published data concerning the susceptibility of *P. falciparum* in French Guiana are not numerous. Dedet et al. (*loc. cit*.) carried out *in vitro* tests in French Guiana in 1988: 91% of the 32 isolates, obtained between 1983 and 1987, were resistant to chloroquine; 40% were resistant to amodiaquine and 17% resistant to quinine, while all the isolates were susceptible to mefloquine. Gay et al. (*loc. cit.*) carried out *in vitro* tests in 1987: the valid tests showed that 66% of 77 isolates were

susceptible to chloroquine. 96% of the isolates were susceptible to quinine and mefloquine. Eight years later in 1995, our study showed that *in vitro* resistance to chloroquine had increased: only 3% of the isolates were susceptible. Considering this result and the increasing number of failures of treatment with chloroquine, a consensus conference held in Cayenne in October 1995 did not recommend chloroquine for the treatment of falciparum malaria. Now the recommended drugs are mefloquine, halofantrine or quinine associated with doxycycline, when these drugs are not contra-indicated.

The different strains have been also classified according to their origin and the stage of the disease (severe and mild cases). All the regions where transmission occurs in French Guiana are represented. However, most of the isolates (24 of the 38) came from the Maroni valley and it was not possible to compare the susceptibility of the isolates according to their origin because of the lack of collection in the other areas of transmission. No significant difference was observed between percentages of suceptibility to quinine in severe or mild cases. Only one isolate was susceptible to chloroquine which came from a severe case. Frequently, the complications of the case are not a consequence of the antimalaria treatment failure because all the cases were rapidly cleared of the parasites.

Further studies are under way to determine the genetics of these strains. The strains will be compared genetically according to the results of the *in vitro* tests, the severity of the cases and the origin of the strains. On the other hand, a new survey is organized to follow the evolution in Oyapock and Maroni valley. Self-medication at subcurative levels with halofantrine as presumptive treatment is widespread among gold-diggers. The Maroni valley situation can change in worse if the level of transmission becomes higher.

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