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RESISTANCE TO *IN VIVO* AND *IN VITRO* CHEMOTHERAPIES IN THE BRAZILIAN AMAZONIA

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The ecological conditions in the Brazilian Amazonia are extremely favorable for the maintenance of endemic malaria due to the constant presence of *A. darlingi* the principal vector of malaria in the Amazonian region. Recent migrations of human populations, principally in the State of Rondônia have contributed to the spread of the disease. Barreto in 1940 documented six million cases of malaria in Brazil. With the intensification of programs to eradicate the disease the extent of the endemic area and number of persons afflicted have diminished. However since 1974 the number of confirmed cases of malaria has gradually risen. In 1974, 66.689 cases of infectious malaria were reported (SUCAM, 1985). By 1984 (SUCAM, 1985) the number of cases rose to 378.257, of which 99.5% were in the Amazon. In 1985, 168.302 cases of malaria were registred in Rondônia alone. One of the factors which has made endemic malaria very difficult to control is the resistance which strains of *P. falciparum* have demonstrated to most anti-malarial drugs.

The indiscriminate use of antimalarial drugs and other factors which influence the *Plasmodium* are probably responsible for the changes occurring in the therapeutic response of *P. falciparum*. We define a strain as resistant when it is capable of surviving and multiplying in spite of receiving a dose of antimalarial drug equal or superior to the levels normally tolerated by a patient.

Reports of resistance to antimalarial drugs date from the last century. In Italy, in 1894 (Peters, 1970), there were reports of *P. falciparum* demonstrating resistance to quinine. The first reference in Brazil to increased resistance of *P. falciparum* to quinine was that made by Miguel Couto in 1908. Oswaldo Cruz in 1910 reported that some patients had to receive larger than normal doses of quinine to cure malaria.

During and after the Second World War chloroquine, a 4-aminoquinoline derivative came to be widely used as an anti-malarial. The first cases of chloroquine resistance in Brazil were reported by Silva et al. in 1961, in a study of patients from Pará, Maranhão (Amazônia). Lopes in 1970 studied *in vivo* chloroquine resistance in strains from Amazônia (Pará, Goiás and Rondônia) and showed resistance at RI, RII and RIII levels. Riechmann & Lopes-Antunâmo in 1971, studying strains from Mato Grosso, demonstrated resistance to chloroquine. In 1972, Sá Filho et al., in Pará administred chloroquine to falciparum malaria patients without producing cure. Alecrim (1981) in a retrospective study carried out from 1974-1979 showed that 58 patients from Amazônia treated with chloroquine showed 100% resistance at the RIII level (Table I). Alecrim et al., in 1986, reviewed patients treated with chloroquine and amodiaquine in SUCAM-AM, to evaluate drug response. 27 patients were treated with chloroquine; 12 (44.4%) of these had strains resistant at the RII level and 6 (18.5%) showed strains resistant at the RIII level.

With the advent of chloroquine resistance, sulfonamides and anti-folates came to be used for therapy of falciparum malaria, with excellent results. In the meanwhile, in 1972, the first reports of resistance to these combinations appeared in the literature.

In Brazil, Almeida et al., in 1972, in a retrospective study from 1966-1972 showed that of 104 patients from Goiás treated with the combination of sulfadoxine and pyrimethamine, five did not show elimination of parasites from the blood. Alecrim (1981) in a retrospective study carried out in patients from Amazônia from 1974-1979 showed that of 164 patients treated with sulfadoxine and pyrimethamine, four showed strains resistant in the RII level (Table II). In the same period, these authors reviewed 430 patients treated with the combination sulfamethoxazole and trimethoprim and found RI resistance in four strains and RII resistance in two (Table III). Silva, in 1978, called attention to the appearance of Fansidar resistance in Maranhão. Alecrim et al., in 1982, published RIII resistance to sulfadoxine and pyrimethamine in a strain from Amazonas (Quatá River). Alecrim 1985 reviewed 52 patients infected with Amazonian strains of *P. falciparum* and treated with sulfadoxine and pyrimethamine; of these 32.7% were RI resistant, 42.3% were RII and 7.7% were resistant at the RIII level (Table IV).

In the face of resistance to 4-aminoquinolenes and sulfamides plus anti-folates, quinine came to be used and clindamycin and mefloquine were tested in multiply resistant strains in Ama-

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zonia. Albuquerque et al. (1985), from 1981-1984, showed the existence of strains RI, RII and RIII to quinine and characterized the diminished response of *P. falciparum in vivo* to quinine in the same period.

Clindamycin, an antibiotic derived from lincomycin, showed itself effective in clearing strains of *P. falciparum* from the blood, including multiply resistant strains. However, as in 1985, we showed that clindamycin was incapable of curing seven of 139 patients accompanied for 35 days (Table V). Mefloquine, a methanol quinoline derivate, in our experience has been able to clear even high *P. falciparum* parasitemias. The observation of 60 patients who received 500 or 750mg of mefloquine, or 750mg of mefloquine plus Fansidar, showed that in five, the parasitemia became positive again during four weeks follow-up (Table V). Boulos et al., in 1986, demonstrated resistance at *P. falciparum* to the combination of Mefloquine and Fansidar at the RI level in patients from Amazônia.

TABLE I

Response of patients infected with *P. falciparum* malaria to Chloroquine from 1974-1979 (retrospective study)

Response to Drug / Year	S		R_I		R_{II}		R_{III}	
	No.	%	No.	%	No.	%	No.	%
1974	0	0,0	0	0,0	0	0,0	5	100,0
1975	0	0,0	0	0,0	0	0,0	8	100,0
1976	0	0,0	0	0,0	0	0,0	16	100,0
1977	0	0,0	0	0,0	0	0,0	13	100,0
1978	0	0,0	0	0,0	0	0,0	7	100,0
1979	0	0,0	0	0,0	0	0,0	9	100,0

TABLE II

Response of patients with *P. falciparum* malaria to the combination of Sulfadoxine and Pyrimethamine from 1974-1979 (retrospective stydy).

Response to Drug / Year	S		R_I		R_{II}		R_{III}	
	No.	%	No.	%	No.	%	No.	%
1974	9	100,0	0	0,0	0	0,0	0	0,0
1975	7	100,0	0	0,0	0	0,0	0	0,0
1976	20	100,0	0	0,0	0	0,0	0	0,0
1977	98	97,0	0	0,0	3	3,0	0	0,0
1978	16	100,0	0	0,0	0	0,0	0	0,0
1979	14	93,4	0	0,0	1	6,6	0	0,0

TABLE III

Response of patients with *P. falciparum* malaria to the combination of Sulfamethoxazole and Trimethoprim from 1974-1979 (retrospective study)

Response to Drug / Year	S		R_I		R_{II}		R_{III}	
	No.	%	No.	%	No.	%	No.	%
1974	5	100,0	0	0,0	0	0,0	0	0,0
1975	22	100,0	0	0,0	0	0,0	0	0,0
1976	67	100,0	0	0,0	0	0,0	0	0,0
1977	103	100,0	0	0,0	0	0,0	0	0,0
1978	155	98,1	2	1,3	1	0,6	0	0,0
1979	78	96,3	2	2,5	1	1,2	0	0,0

TABLE IV

In vivo study. Response of *P. falciparum* to Sulfadoxine and Pyrimethamine (S + P) in strains from the Brazilian Amazonia studied from 1981-1985, in patients followed for 35 days

Response	Sulfadoxine + Pyrimethamine
	%
Sensitive	17,3
R_I	32,7
R_{II}	42,3
R_{III}	7,7

*p = 52

*Number of patients studied

TABLE V

In vivo study. Response of *P. falciparum* to Clindamycin and Mefloquine in patients treated during the period 1980-1985 and followed for 35 days

Response	Clindamycin	Mefloquine
	%	%
Sensitive	94,96	91,7
R_I	5,04	8,3
R_{II}	–	-
R_{III}	–	-
*p = 139		*p= 60

*Number of patients studied

With the ability to culture *P. falciparum* it became relatively easy to carry out drug resistance studies *in vitro*. Riechmann et al., in 1971, demonstrated resistance in *P. falciparum* strains from Mato Grosso. Alecrim et al. (1982) since 1982 have been studying *in vitro* resistance of *P. falciparum* to chloroquine and mefloquine in the Brazilian Amazon. In the case of chloroquine, they studied 168 blood samples from patients from the Brazilian Amazon. 49 tests were carried out with the microtechnique (Table VI) and 119 with the macrotechnique; in both tests resistance to chloroquine was present at high levels (Table VII). In the case of mefloquine, 38 microtests were carried out on samples from patients from Amazônia and 11 resistant strains were identified according to the WHO (1963) criteria (Table VIII).

TABLE VI

In vitro study with Chloroquine: study of 49 strains of *P. falciparum* from the Brazilian Amazonia

Inhibitory Concentration (pmol)	Sample	
	No.	%
1 (pmol)	–	–
2	–	–
4	–	–
5,7	4	8,1
8	2	4,1
16	4	8,1
32	9	18,4
There was no inhibition of schizonts	30	61,3

TABLE VII

In vitro study with Chloroquine: 119 strains of *P. falciparum* from the Brazilian Amazonia

Inhibitory Concentration (n mol)	Sample No.	%
0,25 (n mol)	–	
0,50	--	
0,75	–	
1,00	-	
1,50	7	5,9
2,00	19	15,9
3,00	20	16,8
There was no inhibition of schizonts	73	61,4

TABLE VIII

In vitro study with Mefloquine in 38 blood samples from the Brazilian Amazonia.

Inibitory Concentration	Sample No.	%
0,5 (pmol)	11	29,0
1	2	5,3
2	4	10,5
4	8	21,1
5,7	4	10,5
8	4	10,5
16	2	5,3
There was no inhibition of schizonts	3	7,8

Santos & Rosário, in 1984, published a study on chloroquine and mefloquine in 19 samples from patients from Pará, showing resistance to chloroquine in 12 and 100% sensitivity to mefloquine. Di Santi et al., in 1986, presented *in vitro* studies with chloroquine and mefloquine in 40 samples from patients from Amazônia, finding 100% resistance to chloroquine and 11 samples resistant to mefloquine.

The resistance of *P. falciparum* to antimalarials in Amazônia demands continual surveillance, and the already demonstrated levels of resistance to 4-aminoquinolenes and sulfadoxine plus pyramethamine emphasize the need for other practical, effective drugs in endemic areas. It is urgent that mefloquine be made available in the country, and in order to prevent its careless and indiscriminate use, which would cause loss of effectiveness and its distribution must be controlled by SUCAM.

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