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Research Note

ISOLATION AND CHARACTERIZATION OF A *PLASMODIUM FALCIPARUM* STRAIN: COMPARATIVE STUDY WITH FOUR DESCRIBED STRAINS

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The isolation and characterization of *Plasmodium* strains has been encouraged in the last decade. The knowledge derived from the developed work allowed the proper use of plasmodia componentes in diagnosis and epidemiological studies.

In recent years, biochemical and immunological methods have proved to be useful for identification of genetically different malaria parasites. Studies of isoenzimatic patterns and their relationship with drug resistance permit us to know if isolated parasites with low resistance levels are the result of a mixture of parasitic populations, sensitive and resistant *(WHO/76.50,* p. 339-345). Knowledge about the capability of some strains to be recognized by antibodies present in sera from malaria patients (J. Pividal et al., 1987, *Rev. Cubana Med. Trop., 39:* 7-14) is useful to carry out more specific serological research as well as to know the specific characteristics of the infecting *P. falciparum* strain.

Although the existing literature includes various reports on isolation and characterization of *P. falciparum* strains (D. Walliker, 1985, *Ann. Soc. Belge Med. Trop., 65:* 69-71; V. E. Rosario et al., 1985, *Rev. Inst. Med. Trop. São Paulo, 27:* 274-278), we could not find any concerning a strain from Angola.

In this paper, we report some of the specific characteristics of a strain isolated from a Cuban patient infected in Angola, comparatively to other previously described strains.

Blood samples were collected and stored in liquid nitrogen according to the method of A. W. Rowe et al. (1968, *Cryobiology, 5:* 119-128) until used.

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Parasites were grown by methods derived from that established by W. Trager & J. B. Jensen (1976, *Science, 193:* 673-675). The RPMI-1640 medium was supplemented with 1.19 mM inosine, 10.59 mM sucrose, 40.13 mM TES, 24.99 mM sodium bicarbonate, 2.24 mM L-glutamine, 1.91 mM glutation, 15% AB+ serum for isolation of strains and 10% A+ serum for maintenance *in vitro* culture of the remaining study strains. Strains from lines FCQ-2, (Australia), M-13 (Senegal), M-94 (V. Rosario, 1981, *Science, 212:* 1037-1038) (Thailand) and M-21 (China) were supplied by Dr Lambert of the Department of Pathology CMU, Switzerland.

In vitro chloroquine susceptibility tests were conducted for all strains by a microtechnique according to the methodology described by K. H. Rieckmann et al. (1978, *Trans. R. Soc. Trop. Med. Hyg., 75:* 268-270). Ring stage parasites were obtained by sorbitol synchronization (C. Lambros & J. P. Vanderberg, 1979, *J. Parasitol., 65:* 418-420) and adjusted to a starting parasitaemia of 0.3% in a 5% hematocrit. Results were obtained by microscopic observation of smears in slides stained by the method of Romanosky (1967, *Manual de diagnostico microscopico de la malaria,* Organización Panamericana de la Salud).

Using the Indirect Immunofluorescence assay (IFA) described by A. J. Sulzer (1969, *Am. J. Trop. Med. Hyg., 18:* 199-205) 29 serum specimens from Angola *P. falciparum* malaria patients, were tested against antigenic preparations of each strain.

Conditions of electrophoresis and visualization of areas of enzymatic activity on the starch gel were described by R. Carter (1978, *Parasitology, 76:* 241-267). The enzymes glucose phosphate isomerase (GPI, EC 5.3.1.9), lactate deshidrogenase (LDH, EC. 1.1.1.27) and adenosine deaminase (ADA, EC 3.5.4.4.) were studied.

TABLE

Adaptative sequency of a strain of *Plasmodium falciparum* to *in vitro* culture

Culture in days	Parasites per 10,000 erythrocytes R	T	S	G	Total
0	18	8	–	2	28
2	20	9	7	3	39
4	48	17	23	2	90
4 (1:1)[a]	–	–	–	–	–
6	51	22	45	3	121
8 (1:2)[a]	–	–	–	–	–
10	73	21	64	2	160
12	93	34	88	2	217
14	154	85	39	3	281
14 (1:2)[a]	–	–	–	–	–
16	33	59	78	3	173
18	101	82	89	3	275
18 (1:2)[a]	–	–	–	–	–
20	53	98	105	3	259

R = rings, T = trophozoites, S = schizonts, G = gametocytes.
a: subculture dilution.

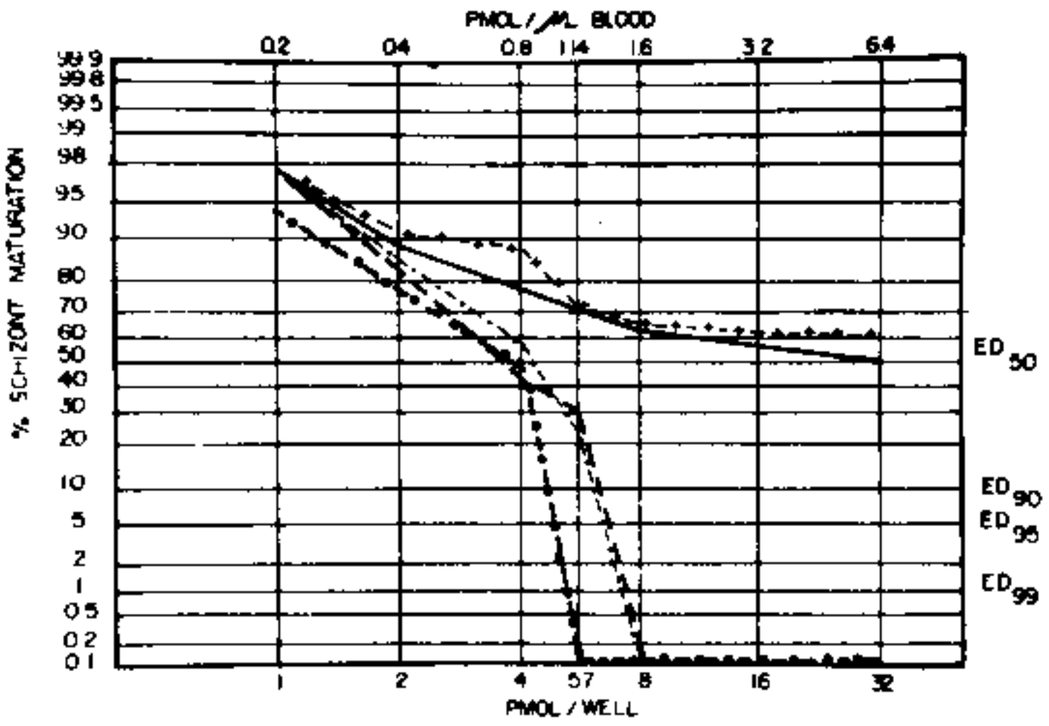

Fig 1: inhibition of schizont maturation of *Plasmodium falciparum* under chloroquine in the *in vitro* microtest. FCK-1/CA (– + –), FCQ-2 (—), M-13 (– – –), M-94 (–.–.–) and M-21 (–.–.–) strains.

The patient from whom the strain was isolated showed resistance to chloroquine and had a parasitaemia of 80 parasites per field at the time of sampling. The strain isolated in our laboratory was termed FCK-1/CA (the first culture line of *P. falciparum* – FC – started at the Institute of Tropical Medicine Pedro Kouri – K – from Cuban patient infected in Angola – CA–) according to the methodology proposed by J. B. Jensen & W. Trager (1979, Am. J. Trop. Med. Hyg., 27: 743-746).

Performance of the strain isolated in our laboratory during the first 20 days of culture is shown in the Table. The isolated strain has

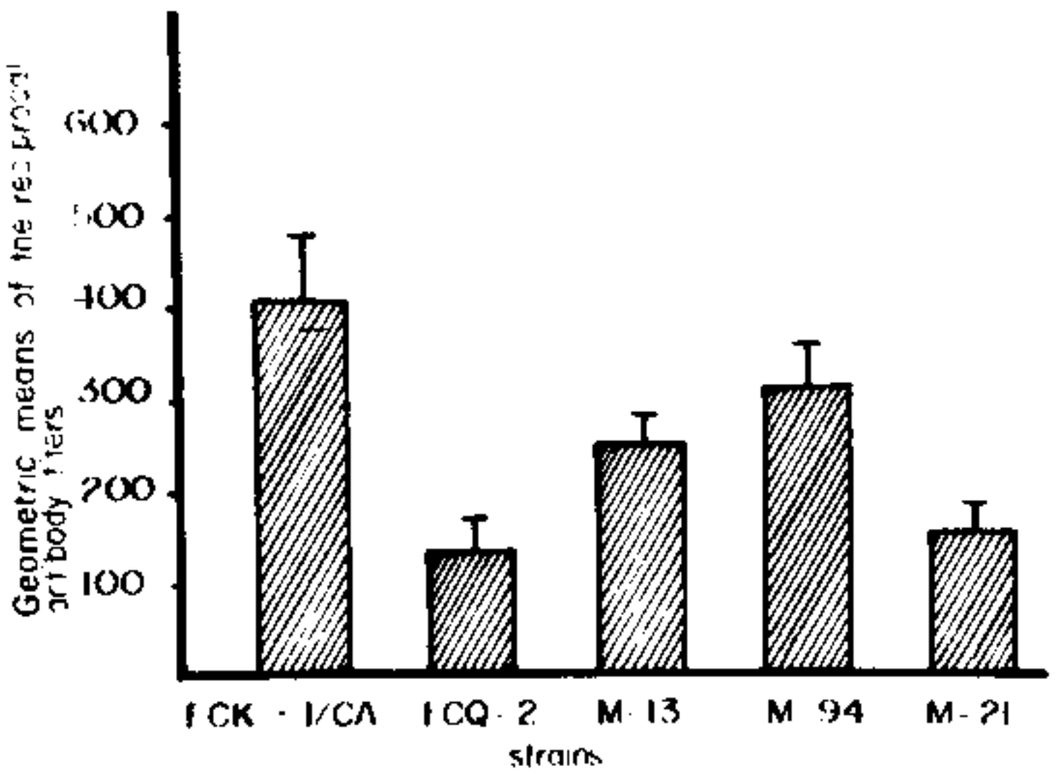

Fig. 2: distribution of the geometric means of the reciprocal titers of antibody against strains of *Plasmodium falciparum* from different geographical origins.

been kept *in vitro* culture for one year, and have been cryopreserved and decryopreserved three times during this period. The presence of gametocytes was demonstrated in this strain.

The understanding of the biology, genetic and drug resistance of parasites, as well as of immunological mechanisms in malaria depend on the knowledge of the individual particularities of parasites which differ between species, strains and even clones. In Fig. 1 the chloroquine sensitivity of FCK - 1/CA is compared to those of the four other strains. A decreased percentage of schizonts is noted in each strain as drug concentration increases, being this effect less marked in FCQ-2 and FCK-1/CA strains, which maintain 50.93 and 60.13% of schizonts respectively in 32 p-mol of chloroquine. In the remaining ones, the percentage of schizonts was null from 5.7 p-mol for M-21 strain and 8 p-mol for M-13 and M-94 strains. ED_{50} for M-13 and M-21 strains was found between 2-4 p-mol, while ED_{90}, ED_{95} and ED_{99} were found between. 5.7-8 and 4-5.7 p-mol respectively. For M-94 strain, ED_{50} was found between 4-5.7 p-mol and ED_{90}, ED_{95}, ED_{99} were found between 5.7-8 p-mol; being coincident with ED_{90}, ED_{95}, ED_{99} of the M-13 strain. Therefore FCK-1/CA (Angola) and FCQ-2 (Australia) differ from the remaining strains in their responses to chloroquine since they have shown a high degree of resistance unlike the other studied strains from Senegal, Thailand and China. Fig. 2 compares the geometric means of the reciprocal antibody titers obtained by IFA. When sera from Angola patients were tested against parasite of each strain a significative difference was re-

corded between the new strain and both FCQ-2; M-21 but not the former, M-13 and M-94, fact that could be explained by the geographical origin of the studied strains. Indeed E. H. Benzerroug et al. (1986, *Am. J. Trop. Med. Hyg., 35:* 255-258) reported that in seroepidemiological studies, sensitivity could be improved by the use of a homologous antigen from the same geographical origin.

Isoenzymatic patterns were the same for all studied strains, and our results were similar to those obtained by A. Sanderson et al. (1981, *Trans. R. Soc. Trop. Med. Hyg., 75:* 263-267) for M-13 and M-21 strains and by S. Thaithong et al. (1984, *Trans. R. Soc. Trop. Med. Hyg., 78:* 242-245) for M-94 strain. Starch gel electrophoresis proved to be an extremely useful tool for differentiation of malaria parasites. However, these differences are less striking in human malaria than in rodent's malaria subspecies (D. Walliker, 1983. Protein polymorphism: Adaptative and Taxonomic Significance, p. 27-33. In G. S. Oxford & D. Rollison (eds) *Enzyme Variation in Malaria Parasite Populations,* Academic Press, London). Although differences in the pattern of response to chloroquine were observed this work did not record any difference in the isoenzymatic pattern of the strains studied. According to Myint-Do et al. (1984, *Trans. R. Soc. Trop. Med. Hyg. 78:* 471-473) the enzyme polymorphism is more frequent in isolates having a high level of resistance to chloroquine but no direct relationship is known between the isoenzymes studied and the level of chloroquine resistance. The present study confirms significant differences regarding antibody response against different antigens and the pattern of response to chloroquine but not the enzyme polymorphism between isolates of separate geographic localities.

In conclusion, FCK-1/CA showed to be as resistant as FCQ-2 strain. Fluorescent titers demostrated significative differences between the new isolated strain and both FCQ-2 and M-21. No differences were established between the new and other strains concerning isoenzimatic patterns.