ANTIMALARIAL ACTIVITY OF *S*-ISOBUTYL ADENOSINE AGAINST *PLASMODIUM FALCIPARUM* IN CULTURE

W. TRAGER
The Rockefeller University, New York, NY, USA

and

M. ROBERT-GERO and E. LEDERER
Institut de Chimie des Substances Naturelles, CNRS, 91190 Gif sur Yvette, France

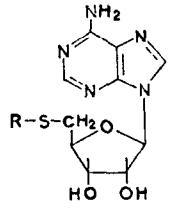
Received 7 November 1977

1. Introduction

The continuous culture of the human malarial parasite *Plasmodium falciparum* [1] provides a convenient new method for testing materials for antimalarial activity. Especially suitable for this purpose is the petri dish technique [2]. This method has now been used to demonstrate a new class of substances with antimalarial action, *S*-isobutyl adenosine (SIBA) and certain of its analogues. These analogues of *S*-adenosyl homocysteine have been shown to inhibit several oncogenic viruses [3–5] and mitogen-stimulated blastogenesis of lymphocytes [6].

2. Materials and methods

Two strains of *P. falciparum* were used, both after prolonged culture in vitro (see Table 1). The parasites were grown in human Type AB erythrocytes in culture medium RPMI 1640 (Grand Island Biol. Co.) supplemented with Hepes buffer at 25 mM and 10% human Type AB serum. For each experiment a single suspension of parasitized cells appropriately diluted with fresh uninfected red cells was used for all the cultures. These were prepared in 35 mm plastic petri dishes, each receiving 1.5 ml 10% red cell suspension. The cultures were incubated at 37°C in a candle jar and fresh medium was provided daily (see [2] for



details). The parasites were exposed to drug either from the outset or after 2 days growth in absence of drug.

The compounds tested were 5′-deoxy-5′-*S*-isobutyl adenosine (SIBA) (where R group attached to the S is $(CH_3)_2CH–CH_2–$) [7], an analogue designated as Iso SIBA [R is $CH_3–CH_2–CH(CH_3)–$] and 2-pyridyl-*S*-adenosine [8] (ANVAR-Institut Pasteur patents pending). Each was dissolved by grinding 3.7 mg in a mortar with 20 ml RPMI 1640 medium containing 25 mM Hepes but without bicarbonate. The solutions, at a concentration of 0.5 mM, were sterilized by filtration through a glass ultrafilter and stored in a refrigerator. Just before use appropriate amounts of sterile 5% $NaHCO_3$ solution and sterile serum were added, and desired dilutions made with complete culture medium.

Counts of the parasites were made from dried films fixed in methanol and stained with Giemsa's stain. A film was always prepared from the suspension used to start the cultures ('0' time) and again

 Elsevier/North-Holland Biomedical Press

after 2 days exposure to drug. Further details are given in table 1 together with the results of 3 experiments, two with SIBA and its analogues and one with chloroquine for purposes of comparison.

3. Results and discussion

For both a Southeast Asian and an African strain, of *P. falciparum* SIBA was markedly inhibitory at 300 μM but not at lower concentrations (table 1, exps. B and C). This is the same as the concentration that gave 94% inhibition of convanavalin A-stimulated blastogenesis of lymphocytes [6], somewhat lower than the concentration effective against Rous sarcoma virus in vitro [3], and higher than the 50 μM concentration inhibiting polyoma virus growth in mouse embryo cells [4]. Iso-SIBA and 2-pyridyl-*S*-adenosine, tested only against the African strain, appeared to be slightly less active than SIBA. These results indicate how in vitro cultures of *P. falciparum* can be utilized for direct screening for new agents with antimalarial activity against the human parasite. It must be recognized, of course, that such screening would

Table 1
Inhibition of *P. falciparum* in vitro by chloroquine and by *S*-isobutyl adenosine and two of its analogues

Exp.	Drug	Concentration (μM)	Parasites/10 000 rbc after 2 days
A	None	–	250, 309, 257 av: 272
	Chloroquine (base)	0.10	319, 327, av: 323
		0.33	344, 291, av: 317
		1.00	116, 105, av: 111
B	None	–	172, 225, 206 av: 201
	SIBA	30	261, 237, 191 av: 230
		100	93, 245, 109 av: 136
		300	17, 25, 23 av: 22
C	None	–	190, 120, 176 av: 162
	SIBA	300	53, 60, 26 av: 46
		180	146, 140, 130 av: 139
	ISO-SIBA	300	73, 74, 81 av: 76
		180	128, 146, 202 av: 158
	2-pyridyl-*S*-adenosine	300	35, 57, 92 av: 61
		180	122, 162, 199 av: 161

[a]Counts for each culture dish and averages (av)

For exps. A and B. strain FVO [13] (chloroquine resistant) was used after it had been one year in continuous culture (now designated as Strain FCR-1). A petri dish culture was diluted with fresh erythrocytes to give an initial parasite count of 10/10 000 red cells and all dishes were prepared from this suspension. They were kept in medium without drug for 2 days. On days 2 and 3, when the medium was changed the controls were again given medium without drug whereas the experimental dishes were given medium with drug incorporated in it to give the indicated concentrations. Slides for counts were made on 4, and hence after 2 days of exposure to drug

For exp. C, strain 6252 (isolated directly in culture from infected human blood brought from Gambia, West Africa, by Friedman, M.) was used after it had been 7 months in culture (now designated Strain FCR-4). A flow vessel culture with a parasitemia of 5% was diluted with fresh erythrocytes to give a suspension with an initial count of 20/10 000 red cells. The experimental dishes were exposed to drug from the outset and slides for counts were made 2 days later

not reveal agents whose activity depends on metabolic transformation by a host organism, nor does it fully obviate the need for drug tests in both infected and uninfected animals. In this connection it is of interest that SIBA has been used in intact mice infected with Friend virus and gave 40% prolongation of life span at a dosage of 1 mg/mouse (Chermann, J. C., Institut Pasteur, personal communication).

Furthermore at 500 μM SIBA had no effect on growth of normal mouse embryo cells [4], and at 1 mM it had only a reversible cytostatic effect on normal chick embryo fibroblasts [3].

Since SIBA is an inhibitor of transmethylases [3,9,10], its antimalarial effect may depend on this activity. Detailed study of the metabolism of *P. falciparum* in the presence of the drug is needed, and here again the in vitro cultures should be invaluable.

It will be noted (table 1, exp. A) that chloroquine had only a moderate inhibitory effect even at 1.0 μM on strain FVO, a known chloroquine-resistant strain. Using short term cultures in *Aotus trivirgatus* erythrocytes [11], the FVO strain was found resistant to chloro quine concentrations up to 0.3 μg/ml (about equivalent to 1 μM). Similarly [12], using short term cultures in human blood the resistant Vietnam-Marks strain was found to be about 50% inhibited by chloroquine at 2–3 μM, whereas 0.2 μM gave the same inhibition of the susceptible Uganda-Palo Alto strain. Effective plasma levels of chloroquine for non-resistant strains of *P. falciparum* are about 0.1 μM. Continuous cultures of *P. falciparum* should be of special use for comparative studies of different strains with regard to relative susceptibility to chloroquine and other drugs.

Acknowledgements

This work was supported in part by a Contract with the US Agency for International Development. The French workers thank the Pasteur Institute for financial help.

References

[1] Trager, W. and Jensen, J. B. (1976) Science 193, 673–675; Trager, W. (1976) in: Biochemistry of Parasites and Host–Parasite Relationships (Van den Bossche, H. ed) pp. 427–434, Elsevier North/Holland Biomedical Press, Amsterdam.
[2] Jensen, J. B. and Trager, W. J. (1978) Parasitol. in press.
[3] Robert-Géro, M., Lawrence, F., Farrugia, G., Berneman, A., Blanchard, P., Vigier, P. and Lederer, E. (1975) Biochem. Biophys. Res. Commun. 65, 1242–1249.
[4] Raies, A., Lawrence, F., Robert-Géro, M., Loche, M. and Cramer, R. (1976) FEBS Lett. 72, 48–52.
[5] Jacquemont, B. and Huppert, J. (1977) J. Virol. 22, 160–167.
[6] Bona, C., Robert-Géro, M. and Lederer, E. (1976) Biochem. Biophys. Res. Commun. 70, 622–629.
[7] Hildesheim, J., Hildesheim, R. and Lederer, E. (1971) Biochimie 56, 1067–1071.
[8] Legraverend, M., Ibanez, S., Blanchard, P., Enouf, J., Lawrence, F., Robert-Géro M. and Lederer, E. (1977) Eur. J. Med. Chem. 12, 105–108.
[9] Hildesheim, J., Hildesheim, R., Yon, J. and Lederer, E. (1972) Biochimie 54, 989–995.
[10] Hildesheim, J., Goguillon, J. F. and Lederer, E. (1973) FEBS Lett. 30, 177–180.
[11] Siddiqui, W. A., Schnell, J. V. and Geiman, Q. M. (1972) Am. J. Trop. Med. Hyg. 21, 392–399.
[12] Rieckman, K. Y., McNamara, J. V., Frischer, H., Stockert, T. A., Carson, P. E. and Powell, R. D. (1968) Am. J. Trop. Med. Hyg. 17, 661–671.
[13] Rieckmann, K. H. (1971) J. Am. Med. Assoc. 217, 573–578.
[14] Geiman, Q. M. and Meagher, J. (1967) Nature 215, 437.