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THERAPEUTIC ACTIVITY OF SOME SULFONAMIDE COMPOUNDS ON NORMAL AND CHLOROQUINE-RESISTANT STRAINS OF *PLASMODIUM BERGHEI*

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SUMMARY

There has been studied the comparative activity of some sulfonamide compounds on one normal and one chloroquine-resistant strain of *Plasmodium berghei*. The 40-fold chloroquine-resistant strain proved to be clearly more sensitive to the sulfonamide compounds than the normal strain. The indexes of resistance, based on the ED_{50} and ED_{90} (doses reducing parasitemias in 50 and 90 per cent as compared with those of the untreated controls) of both strains were consistently lower than 0.7, which shows that the resistant strain was hypersensitive to sulfonamide compounds.

INTRODUCTION

It has been demonstrated that chloroquine-resistant strains of *P. berghei* may exhibit marked cross-resistance to some compounds as quinine and its derivatives or may still show normal sensitivity to other active antimalarial drugs as pyrimethamine (JACOBS [3], PETERS [4], HERRERO [2], HAWKING & GAMMAGE [1]). As regards sulfonamide compounds, HAWKING & GAMMAGE [1] reported that a chloroquine-resistant strain of *P. berghei* proved normally sensitive to sulfadiazine. PETERS [4] observed that a chloroquine-resistant strain displayed some hypersensitivity towards sulfadiazine. More recently, RICHARDS [6] demonstrated that no difference could be found between the optimum dose levels of sulphorthomidine for normal and chloroquine-resistant strains of *P. berghei*.

In this paper the comparative action of some sulfonamide compounds on a normal and a 40-fold chloroquine-resistant strain of *P. berghei* has been described.

MATERIAL AND METHODS

Drugs used

The following drugs have been used:

a) Chloroquine diphosphate

b) 4-Sulfanilamido-2,6-dimethoxy-pyrimidin (Sulfadimethoxine)

c) 4-Sulfanilamido-5,6-dimethoxy-pyrimidin (Sulformethoxine)

d) 2-Sulfanilamido-pyrimidine (Sulfadiazine)

e) Ro 4-8600 (derivative of sulformethoxine)

f) Ro 6-1132 (derivative of sulformethoxine)

Inoculation of animals and counting of parasites

Albino mice weighing 18-20 g were employed. The normal *P. berghei* strain has been kept in the laboratory for 8 years

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through weekly blood passages. In the experiments here described the animals were inoculated with 10^7 infected red cells per 20 g by intraperitoneal route.

The percentage of infected red cells was determined by counting 300-400 unselected cells in blood smears stained by May-Grünwald-Giemsa method, the number of red cells per cubic millimeter in the blood pool being determined by means of a Neubauer chamber.

Production of chloroquine resistance

The method employd to induce chloroquine resistance was based on Peters' observation (PETERS [4]). Chloroquine was administered, by oral route, once a day for four consecutive days from the day after inoculation on. The mice were examined on the 6th or 7th day after inoculation and, when showing parasitemias higher than 3%, were sacrificed and their blood inoculated into normal mice. The first dose selected corresponded to the ED_{50} determined for the normal strain. The other doses were gradually increased, two doses having been used in each experiment: the dose used in the previous treatment (which had not been able to clear the infected animals) and a higher one. In each passage three groups of 5 animals were used: the two groups treated with the different dosages of chloroquine and a control group inoculated with the same inoculum but left untreated.

On the first experiments, when the parasitemias after the chloroquine treatment were rather low, the inoculum, as suggested by PETERS [4], consisted of blood from both treated and untreated animals so that a suitable number of parasites might be inoculated.

Therapeutic activity of sulfonamides

Albino mice weighing 18-20 g were inoculated, by intraperitoneal route, with 10^7 red cells infected with the strain resistant to 150 mg/kg and then divided into five groups. Four groups (of at least 5 animals each) were treated with different doses of the sulfonamide compounds, the highest dose corresponding to the ED_{50} previously determined for the normal strain. A group of ten infected animals were taken as untreated controls. The drugs were administered by oral route, once a day, for four consecutive days, beginning on the day after inoculation.

Other five groups of albino mice were similarly infected with the normal strain and then treated with the sulfonamide compounds according to the same schedule described above.

Determination of ED_{50} and ED_{90}

On the 5th day after inoculation smears were prepared and stained by May-Grünwald-Giemsa technique. The percentage of average parasitemia reduction in the treated animals as compared with the untreated controls was then determined. The ED_{50} (dose which reduces in 50% the parasitemia of the treated animals as compared with that of the untreated controls) was graghically determined by plotting the data on probit-activity log-dose paper. The ED_{90} was similarly determined. The ED_{50} and the ED_{90} were determined by using data from at least two experiments.

Index of resistance

This index was determined as described by PETERS [4]:

$$I.R. = \frac{ED_{50} \text{ or } ED_{90} \quad RC \text{ strain}}{ED_{50} \text{ or } ED_{90} \quad N \quad \text{strain}}$$

RESULTS

Chloroquine resistance

After 15 passages and repeated treatment with gradually increasing doses of chloroquine, for a period of 95 days, the normal strain developed marked resistance to this drug, parasitism being not practically affected by such high doses as 150 and 200 mg/kg, which corresponds, respectively, to 30 and 40 times the normal ED_{50} (Fig. 1). The experiments with the sulfonamide compounds were performed with the chloroquine-resistant strain from the 18th passage on, thas is, when the strain was already steadily resistant to doses up to 150 mg/kg.

Some characteristics of chloroquine-resistant strains previously described by other

Authors have also been detected in our strain. The mortality rate was significantly lower than that of the normal strain, the parasitemia progressed more slowly and pigment was found to be absent in the erythrocytic forms.

Therapeutic activity of sulfonamides

Table I shows the ED_{50} and the ED_{90} (obtained through the administration of five sulfonamide compounds) of mice infected with a chloroquine-resistant and a normal strain of *P. berghei* as well as the resistance indexes. On all experiments the chloroquine-resistant strain proved to be clearly more sensitive to the sulfonamide compounds than the normal strain, the index of resistance being always < 1.

DISCUSSION

P. berghei may be rendered resistant to chloroquine by using different methods as has been shown by several Authors. HAWKING & GAMMAGE [1] used ethyl palmitate intravenous injection to supress phagocytic function, the inoculated animals being then placed on a diet containing 0.005% of chloroquine. This way the *P. berghei* strain became resistant to the maximum tolerated dose of chloroquine. PETERS [4] treated infected mice with increasing doses of chloroquine, parenterally, passages being then performed when the parasite density had increased, at least, 1% after treatment. After a period of 5 1/2 months the parasite resistance was fully developed and they could tolerate a higher dose of the drug than their hosts. JACOBS [3] used increasing oral doses of chloroquine and subsequent passages with the blood of treated animals, resistance to the maximum tolerated dose being reached after the 11th passage (77 days). In our experience a 10-fold resistance was reached after the 12th passage (70 days), the strain having achieved full resistance after the 15th passage (95 days). By the time these experiments were performed, the *P. berghei* strain was already resistant to the toxic dose of 200 mg/kg. On account of the instability of the chloroquine-resistance phenomenon

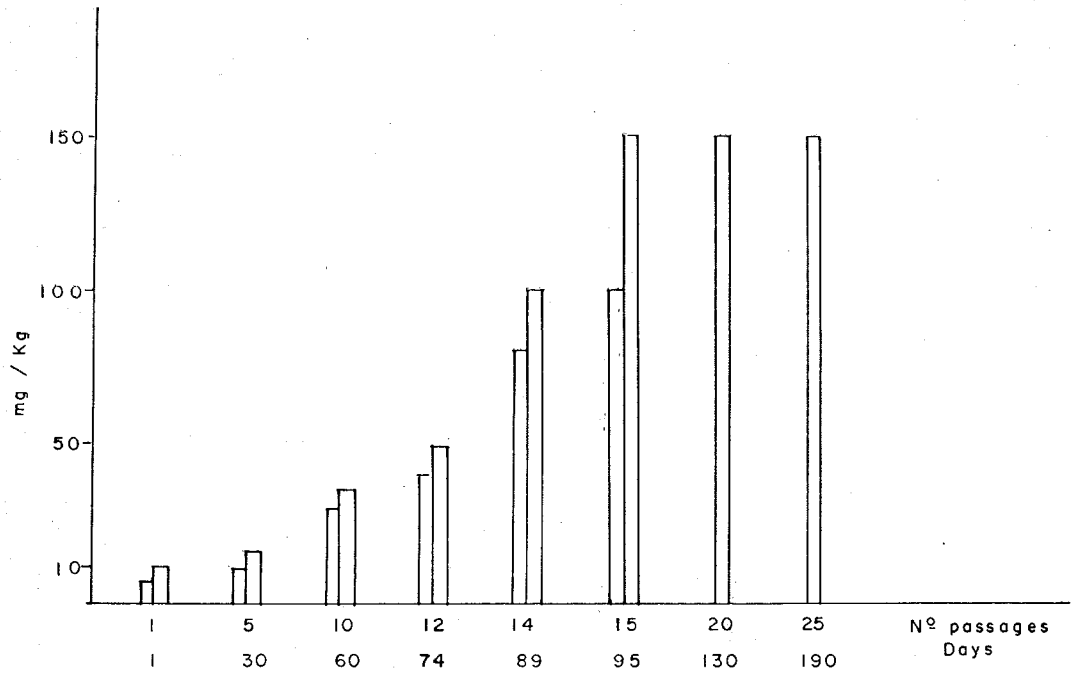

Fig. 1 — Number of passages and chloroquine doses employed to produce a 30-fold chloroquine-resistant strain of *Plasmodium berghei* in the albino mouse

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TABLE I

ED_{50}, ED_{90} and resistance indexes determined in mice infected with a chloroquine-resistant and a normal strain of *P. berghei* and treated with sulfonamide compounds

Sulfonamide compounds	Normal strain		Resistant strain		I.R.$_{50}$	I.R.$_{90}$
	$ED_{50} \pm 2s$	$ED_{90} \pm 2s$	$ED_{50} \pm 2s$	$ED_{90} \pm 2s$		
Sulformethoxine	0.22 ± 0.02	1.15 ± 0.34	0.15	0.70 ± 0.01	0.7	0.6
Sulfadimethoxine	0.50	1.45 ± 0.07	0.30	0.85 ± 0.1	0.6	0.7
Sulfadiazine	0.40 ± 0.15	0.95 ± 0.07	0.18 ± 0.07	0.60 ± 0.1	0.4	0.6
Derivative of sulformethoxine (Ro 6-1132)	0.47 ± 0.07	1.00	0.23 ± 0.07	0.48 ± 0.07	0.5	0.5
Derivative of sulformethoxine (Ro 4-8600)	0.45 ± 0.07	1.25 ± 0.7	0.27 ± 0.1	0.52 ± 0.03	0.6	0.4

and of the possible event of the parasite losing its resistance, the strain used in the present investigation was constantly submitted to the action of 150 mg/kg of chloroquine.

As regards the sensitivity of *P. berghei* chloroquine-resistant strains to sulfonamide compounds, some discrepancies have been detected in the literature. According to HAWKING & GAMMAGE [1] his resistant strain was normally sensitive to sulfadiazine. Another strain of *P. berghei*, rendered highly resistant to chloroquine (60-fold) displayed some hypersensitivity towards sulfadiazine and diamino-diphenyl-sulfone (PETERS [5]). On the other hand, RICHARDS [6] could see that no difference existed between the optimum dose levels of some sulfonamides against normal and chloroquine-resistant strains. More recently, THOMPSON [7] et al. could detect clear hypersensitivity to sulfadiazine and DDS in a *P. berghei* resistant strain.

Our data show the chloroquine-resistant strain to be clearly hypersensitive to the tested sulfonamide compounds, the "index of resistance" being consistently lower than 0.7.

It is difficult to account for the described discrepancies regarding the action of sulfonamides. Differences in the criteria employed by the various Authors are not so significant as to explain the lack of agreement. Despite WARHURST & KILLICK-KENDRICK's [8] description of the spontaneous resistance of a newly isolated strain of *Plasmodium berghei yoelii* to chloroquine, no evidence of sensitivity being affected by the number of blood passages in laboratory has so far been given (PETERS [5]). The degree of chloroquine-resistance does not seem to be the main factor responsible for the difference in behaviour of the resistant strains in the presence of sulfonamides. In fact, THOMPSON et al. [7] have detected hypersensitivity in strains apparently less resistant to chloroquine than those employed by PETERS [4] and by us. It should, however, be emphasized that the experiments dealing with this particular aspect of the chemotherapy of malaria have been carried out under different laboratory conditions, which may explain some of the mentioned discrepancies.

RESUMO

Atividade terapêutica de algumas sulfonamidas em amostras de Plasmodium berghei *normais e resistentes à cloroquina*

A atividade terapêutica de algumas sulfonamidas foi estudada comparativamente em camundongos experimentalmente inoculados com uma cepa normal e uma cepa cloroquina-resistente de *Plasmodium berghei*. Fo-

ram calculados, com ambas as cepas, o ED_{50} e o ED_{90}, isto é, as doses que reduzem, respectivamente, de 50% e de 90% as parasitemias dos animais tratados em relação aos contrôles. Os índices de resistência IR, calculados através da relação entre o ED_{50} ou ED_{90} da cepa resistente e os da cepa normal, foram sempre menores que 0,7 o que traduz hipersensibilidade da cepa cloroquina-resistente às diferentes sulfonamidas.

REFERENCES

1. HAWKING, F. & GAMMAGE, K. — Chloroquine resistance produced in *Plasmodium berghei*. *Trans. Roy. Soc. Trop. Med. Hyg.* **56**:263, 1962.

2. HERRERO, J. — The use of long acting sulfonamides, alone or with pyrimethamine, in malaria (with special reference to sulfometoxine). *Rev. Soc. Brasil. Med. Trop.* **1**: 103-136, 1967.

3. JACOBS, R. L. — Selection of strains of *Plasmodium berghei* resistant to quinine, chloroquine and pyrimethamine. *J. Parasit.* **51**:481-482, 1965.

4. PETERS, W. — Drug resistance in *Plasmodium berghei* Vincke and Lips, 1948. I — Chloroquine resistance. *Exp. Parasit.* **17**:80-89, 1965.

5. PETERS, W. — Rational methods in the search for antimalarial drugs. *Trans. Roy. Soc. Trop. Med. Hyg.* **61**:400-411, 1967.

6. RICHARDS, W. H. G. — Antimalarial activity of sulphonamides and a sulphone, singly and in combination with pyrimethamine, against drug resistant and normal strains of laboratory Plasmodia. *Nature* (London) **212**:1494-1495, 1966.

7. THOMPSON, P. E.; OLSZEWSKT, B.; BAYLES, A. & WAITZ, A. — Relations among antimalarial drugs: results of studies with cycloguanil —, sulfone —, or chloroquine-resistant *Plasmodium berghei* in mice. *Amer. J. Trop. Med. & Hyg.* **16**:133-145, 1967.

8. WARHURST, D. C. & KILLICK KENDRICK, R. — Spontaneous resistance to chloroquine in a strain of rodent malaria (*Plasmodium berghei yoelii*. *Nature* (London) **213**:1048-1049, 1967.

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