

a 61561



WHO/Mal/248 ✓
11 December 1959

ORIGINAL: ENGLISH

STUDIES ON THE SPOROICIDAL EFFECT OF SINGLE DOSES OF
PYRIMETHAMINE - ALONE OR ASSOCIATED WITH CHLOROQUINE -
IN P. VIVAX AND P. FALCIPARUM MALARIA

by

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Investigations carried out at the Malaria Therapy Centre, Berceni,
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I. Experiments with P. vivax

Three strains of P. vivax were used: "K", "Mr" and "V". The "K" strain was obtained from a case of relapsing Korean vivax malaria and was at its thirty-sixth transfer by blood inoculation at the onset of the trials. The strain is said to show no essential differences from local P. vivax strains as regards virulence, immunological characteristics, gametocyte production and infectivity to A. atroparvus.

The two other strains are local Romanian strains obtained from primary infections detected in the field. At the beginning of the experiments they had been transferred by blood inoculation 15 ("Mr" strain) and 7 ("V" strain) times.

Eleven neurosyphilitic patients were infected with P. vivax by inoculation of sporozoites: nine with the "K" strain, one with the "Mr" strain, and one with the "V" strain. Treatment was given 3-14 days after gametocytes had appeared: two patients received a single dose of pyrimethamine (25 mg and 50 mg), and nine patients a single combined dose consisting of 25 mg of pyrimethamine and 300 or 600 mg of chloroquine base.

¹ This investigation was supported by a grant in aid assigned to the Institute of Microbiology, Parasitology and Epidemiology "Dr I. Cantacuzino", Bucarest, by the World Health Organization.

Effect of treatment on the persistence of fever, trophozoites and gametocytes were recorded; infectivity of gametocytes to mosquitos was determined before treatment and again at various intervals after treatment by allowing A. atroparvus to feed on the patients.

Results show that of the mosquitos which had fed on the patients 1-24 hours before drug administration from 20 to 100% became infected (as shown by the presence of oocysts) while all those which fed 4, 8 or 24 hours after treatment failed to become infected (absence of oocysts). In four cases feedings were repeated two or three days after drug administration and none of the mosquitos became infected. The latest feedings were made seven days after treatment (two cases), when gametocytes were either absent or "very few". Only a small number of mosquitos (four and six respectively) were dissected for oocysts and all were found negative.

Details of these experiments are shown in Table I.

II. Experiments with P. falciparum

The "R-Fil" strain of P. falciparum was used. This Romanian strain had been isolated from a relapse in a residual "micro-focus" of infection. Prior to the experiments described here this strain had been transferred 15 times by blood inoculation.

A total of 15 patients were infected with this strain: 10 by blood inoculation and five by inoculation of sporozoites.

The single-dose treatment was administered 5-24 days after the appearance of gametocytes. Five patients received a single dose of pyrimethamine alone (25 or 50 mg) and 10 patients a single combined dose of 25 mg of pyrimethamine + 300 or 600 mg of chloroquine base.

Batches of A. atroparvus which fed on the patients 1-24 hours before drug administration became infected in percentages varying from 11.5 to 72.7%; only in one case (No. 14) the percentage infected was 100%.

None of the mosquitos which fed on the patients 24 hours after treatment became infected. In some cases mosquitos were fed already 2, 3 or 4 hours after drug administration and did not become infected.

Mosquito feedings were repeated at different intervals after treatment: after approximately one week (14 cases), two weeks (seven cases), and after three weeks (three cases). None of these mosquitos was found infected.

Details of these experiments are shown in Table II.

TABLE I. SPORONTICIDAL AND SCHIZONTICIDAL EFFECT OF A SINGLE DOSE OF PYRIMETHAMINE - ALONE OR COMBINED WITH CHLOROQUINE - IN SPOROZOITE-INDUCED P. VIVAX MALARIA ("K", "M" AND "V" STRAINS)

| Case No. (and strain) | Treatment | | | Mosquito feedings | | Mosquito dissections | | Disappearance of: | | Period of observation (days after treatment) | |
|-----------------------|-----------------|-----|---------------|-------------------|---------------------------------------|---------------------------------|----------------|-------------------|-------------------------------------|---|-------------|
| | Days of patency | | Pyrimethamine | Chloroquine | Before (-) & after (+) drug admin. | Gametocytes per mm ³ | No. diss. | % infect.* | Trophozoites (days after treatment) | | Gametocytes |
| | Troph. | Ga. | | | | | | | | | |
| 1 B.V. "K" | 11 | 6 | 50 mg | - | - 4 days + 24 hours + 7 days | 400 "few" 0 | 22 6 6 | 31.8 0 0 | 3 7 | 120 | |
| 2 D.I. ("K") | 12 | 8 | 25 mg | - | - 2 days + 3 days + 7 days | 400 "few" "very few" | 10 8 4 | 40.0 0 0 | 3 8 | 96 | |
| 3 B.I. ("K") | 10 | 7 | 25 mg | 600 mg | - 24 hours + 24 hours + 3 days | 400 100 "very few" | 48 6 10 | 25.0 0 0 | 2 5 | 106 | |
| 4 C.G. ("K") | 16 | 12 | 25 mg | 600 mg | - 24 hours + 4 hours + 8 hours | 400 "few" 273 | 28 20 10 | 20 0 0 | 1 2 | 138 + 104 | |
| 5 V.S. ("K") | 9 | 6 | 25 mg | 600 mg | - 24 hours + 4 hours + 24 hours | 300 284 349 | 27 37 28 | 100 0 0 | 2 3 | 311 (relapse 220 days after first dose, 91 days after second dose) | |

* Presence of oocysts

TABLE I. SPORONTICIDAL AND SCHIZONTICIDAL EFFECT OF A SINGLE DOSE OF PYRIMETHAMINE - ALONE OR COMBINED WITH CHLOROQUINE - IN SPOROZOITE-INDUCED P. VIVAX MALARIA ("K", "Mr" AND "v" STRAINS) (continued)

| Case No. (and strain) | Treatment | | | Mosquito feedings | | Mosquito dissections | | Disappearance of: | | Period of observation (days after treatment) | |
|-----------------------------|-----------------|-----|---------------|-------------------|---------------------------------------|---------------------------------|----------------|-------------------|-------------------------------------|--|--|
| | Days of patency | | Pyrimethamine | Chloroquine | Before (-) & after (+) drug admin. | Gametocytes per mm ³ | No. diss. | % infect.* | Trophozoites (days after treatment) | | Gametocytes |
| | Troph. | Ga. | | | | | | | | | |
| 6 O.R. ("K") | 12 | 7 | 25 mg | 600 mg | - 24 hours + 4 hours + 24 hours | 420 114 194 | 42 42 50 | 100 0 0 | 2 | 4 | (relapse 215 days after first dose, 43 days after second dose) |
| 7 V.F. ("K") | 16 | 14 | 25 mg | 300 mg | - 24 hours + 4 hours + 24 hours | 121 95 96 | 38 30 26 | 100 0 0 | 1 | 3 | (relapse 272 days after first dose, 85 days after second dose) |
| 8 R.A. ("K") | 13 | 10 | 25 mg | 300 mg | - 1 hour + 24 hours + 3 days | 925 743 54 | 34 25 16 | 29 0 0 | 2 | 4 | 147 |
| 9 D.F. ("K") | 7 | 3 | 25 mg | 300 mg | - 1 hour + 24 hours | 464 218 | 20 30 | 85 0 | 2 | 4 | 22 |
| 10 V.N. ("Mr" strain) | 9 | | 25 mg | 300 mg | - 1 hour + 24 hours | 445 80 | 40 40 | 34.3 0 | 2 | 4 | 25 |
| 11 D.G. ("v" strain) | 10 | | 25 mg | 300 mg | - 2 hours + 24 hours + 2 days | 380 198 50 | 37 21 27 | 100 0 0 | 3 | 4 | 32 |

* Presence of oocysts

TABLE II. SPORONTICIDAL AND SCHIZONTICIDAL EFFECT OF A SINGLE DOSE OF PYRIMETHAMINE - ALONE OR COMBINED WITH CHLOROQUINE - IN BLOOD- OR SPOROZOITE-INDUCED *P. FALCIPARUM* MALARIA ("R-Fil" STRAIN)

| Case No. | Treatment | | | Mosquito feedings | | Mosquito dissections | | Disappearance of trophozoites (days after treatment) | |
|-----------------------------------|-----------------|----|---------------|-------------------|---|---|----------------------------------|--|-----------|
| | Days of patency | | Pyrimethamine | Chloroquine | Before (-) & after (+) drug admin. | Gametocytes per mm ³ | No. diss. | | % infect. |
| | Troph. | Ga | | | | | | | |
| 1 L.I. (blood-induced) | 34 | 24 | 50 mg | - | - 24 hours + 24 hours + 4 days + 14 days | 108 146 175 441 | 45 30 14 20 | 17.2 0 0 0 | 3 |
| 2 C.E. (sporozoite-induced) | 23 | 14 | 25 mg | - | - 24 hours + 24 hours + 7 days | "few" "few" "very few" | 42 36 26 | 17.5 0 0 | 3 |
| 3 D.T. (sporozoite-induced) | 19 | 9 | 25 mg | - | - 24 hours + 24 hours + 7 days + 14 days | 60 "few" "few" "very few" | 32 18 28 28 | 72.7 0 0 0 | 2 |
| 4 C.T. (blood-induced) | 17 | 10 | 25 mg | 300 mg | - 24 hours + 24 hours + 11 days | 175 326 82 | 52 38 16 | 24.2 0 0 | 2 |
| 5 D.B. (blood-induced) | 15 | 7 | 25 mg | 600 mg | - 24 hours + 24 hours + 7 days | 163 233 "few" | 22 26 22 | 18.5 0 0 | 3 |
| 6 I.N. (blood-induced) | 14 | 7 | 25 mg | 300 mg | - 24 hours + 4 hours + 24 hours + 6 days + 13 days + 21 days | 676 582 463 159 242 "very few" | 46 47 36 44 30 24 | 68.7 0 0 0 0 0 | 2 |

TABLE II. SPORONTOCIDAL AND SCHIZONTOCIDAL EFFECT OF A SINGLE DOSE OF PYRIMETHAMINE - ALONE OR COMBINED WITH CHLOROQUINE - IN BLOOD-OR SPOROZOITE-INDUCED P. FALCIPARUM MALARIA ("R-F11" STRAIN) (continued)

| Case No. | Treatment | | | | Mosquito feedings | | Mosquito dissections | | Disappearance of trophozoites (days after treatment) |
|------------------------------------|-----------------|----|---------------|-------------|--|---|----------------------------|------------------------|--|
| | Days of patency | | Pyrimethamine | Chloroquine | Before (-) & after (+) drug admin. | Gametocytes per mm ³ | No. diss. | % infect. | |
| | Troph. | Ga | | | | | | | |
| 7 B.A. (sporozoite-induced) | 15 | 5 | 25 mg | 300 mg | - 24 hours + 24 hours + 7 days | 136 383 160 | 47 32 38 | 18.4 0 0 | 2 |
| 8 B.C. (sporozoite-induced) | 22 | 11 | 25 mg | 300 mg | - 24 hours + 24 hours + 7 days | 50 39 13 | 53 28 30 | 11.5 0 0 | 2 |
| 9 Z.N. (blood-induced) | 20 | 11 | 25 mg | 300 mg | - 1 hour + 24 hours + 7 days + 14 days + 21 days | 210 158 104 "very few" "very few" | 37 20 32 18 13 | 59 0 0 0 0 | 2 |
| 10 C.P. (blood-induced) | 30 | 21 | 50 mg | - | - 1 hour + 24 hours + 10 days | 189 177 272 | 38 25 30 | 34.4 0 0 | 4 |
| 11 A.B. (blood-induced) | 17 | 10 | 25 mg | 300 mg | - 1 hour + 24 hours + 8 days | 116 68 72 | 28 25 20 | 23.8 0 0 | 2 |
| 12 V.S. (sporozoite-induced) | 16 | 8 | 25 mg | 300 mg | - 1 hour + 3 hours + 24 hours + 7 days | 1 460 934 788 61 | 26 26 26 16 | 59 0 0 0 | 4 |

TABLE II. SPORONTOCIDAL AND SCHIZONTOCIDAL EFFECT OF A SINGLE DOSE OF PYRIMETHAMINE - ALONE OR COMBINED WITH CHLOROQUINE - IN BLOOD-OR SPOOROZOITE-INDUCED P. FALCIPARUM MALARIA ("R-F11" STRAIN) (continued)

| Case No. | Treatment | | | | Mosquito feedings | | Mosquito dissections | | Disappearance of trophozoites (days after treatment) |
|-------------------------------|-----------------|----|---------------|-------------|---|--|----------------------------------|------------------------------|--|
| | Days of patency | | Pyrimethamine | Chloroquine | Before (-) & after (+) drug admin. | Gametocytes per mm ³ | No. diss. | % infect. | |
| | Troph. | Ga | | | | | | | |
| 13 M.P. (blood-induced) | 13 | 5 | 25 mg | 300 mg | - 1 hour + 24 hours + 8 days | 939 1 278 192 | 40 46 20 | 31 0 0 | 3 |
| 14 O.R. (blood-induced) | 16 | 9 | 50 mg | - | - 24 hours + 2 hours + 24 hours + 7 days + 14 days + 21 days | 805 454 362 225 96 "very few" | 44 20 56 66 27 27 | 100 0 0 0 0 0 | 3 |
| 15 C.I. (blood-induced) | 20 | 9 | 25 mg | 300 mg | - 1 hour + 24 hours + 10 days + 15 days | 2 483 1 929 506 136 | 50 40 12 28 | 70.9 0 0 0 | 2 |

Conclusions

The effect on gametocytes of stated single doses of the drugs used in these experiments was pronounced in the case of P. vivax: the sexual parasites disappeared within a mean of 3-6 days after a single dose of pyrimethamine plus chloroquine or within 7-8 days after a single dose of pyrimethamine only.

The results of experiments on the sporontocidal effect of pyrimethamine indicate that single doses of 25 or 50 mg of this drug render gametocytes of P. vivax and of P. falciparum non-infective to mosquitos within 2-4 hours after administration. It appears that the sporontocidal effect persists for up to three weeks in the case of P. falciparum and, in the case of P. vivax, for up to seven days, viz. as long as gametocytes are present.

Editor's note: Although in the investigation of the sporontocidal effect of pyrimethamine the gametocyte densities after the seventh day were generally low and the numbers of dissected mosquitos small, the results obtained by the authors confirm the findings of a number of other workers (Shute & Maryon, 1954; Jeffery, Young & Eyles, 1956; Young & Burgess, 1957 and 1959; Bray et al., 1959).

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