
This work deals with the life cycle of Plasmodium and in particular ookinete formation. This process is as follows:

A zygote is formed by the fertilization of a macrogamete by a microgamete. The cytoplasm of the zygote shows a distinct movement. Starting from the fourth hour of cultivation, the cytoplasm gradually thrusts out a fine stick-shaped or finger-like projection which continually stretches forward, becomes larger and finally forms a banana-like projection connected to the body of the zygote by a filament.

The nucleus moves gradually towards the projection, then stretches, becomes thinner, enters the banana-like projection through the filament, and moves to the centre of the projection.

By the time the cytoplasm has moved away from the body of the zygote, the body portion has contracted, becoming gradually smaller.

A part of the malaria pigment moves with the cytoplasm into the projection. However, when the nucleus is blocking up the passage to the projection, the remaining malaria pigment is retained in the contracted body of the zygote and gradually collects together until the nucleus has completely entered the projection and then the remaining pigment finally enters into the projection as well.

After the whole content of the zygote has moved into the banana-like projection, the body of the zygote and the connecting filament disappear. The parasite then becomes typically banana-like and is known as a mature ookinete. The mature ookinetes may be observed within 7, 8, 10, 12 or 18 hours after fertilization.


A series of 2,4-diamino-6-substituted piperazinyl-quinazolines was synthesized. The key intermediates of the substituted piperazino-2-aminobenzonitriles were synthesized from m-chlorobenzonitrile by nitration, reacting with piperazine, then reduced and condensed with various substituted halogen compounds. They were cyclized smoothly with cyanoguanidine to form 2,4-diamino-6-substituted piperazinyl-quinazolines. The latter could also be prepared by condensation of 2-nitro-5-chlorobenzonitrile with the substituted piperazines, followed by reduction and cyclization with cyanoguanidine.
After primary screening tests on infected mice, it was found that among these compounds four (compounds X1, 2, 3, 8) showed a suppressive effect on *Plasmodium berghei*, and three (X1, 8, 10) possessed a causal prophylactic activity against *P. yoelii*. They were also screened for sporontocidal activity in the *P. gallinaceum*-Aedes albopictus system, with the result that the sporogony of the parasite was inhibited in 70% of the infected mosquitoes by two compounds (X8, 9) at a concentration of 0.01%.


One chick, weighing 1 kg, was inoculated intravenously with the erythrocytic stages of *Plasmodium gallinaceum*. When parasitaemia reached approximately 40%, a batch of *Aedes albopictus* (i.e. the control group) was allowed to feed on the chick. Then nitroquine was given to the chick in a single oral dose of 2 mg/kg body weight. Another batch of mosquitoes (i.e. the test group) was then allowed to feed on the same chick three hours later.

Slides of ookinetes were stained with Giemsa-colophonium and slides of oocysts were stained either with Giemsa or by the Feulgen method.

The average number of ookinetes per midgut in the test group 24 hours after the blood meal was 958.6, while that of oocysts on the sixth day after infection was 32.7, thus accounting for only 3.3% of the ookinetes. At 34 and 48 hours after the blood meal, there was a great number of ookinetes adhering to the peritrophic membrane in both the test and control groups. In the test group, 90% of the ookinetes in the midgut cavity appeared shrunken and stiff with condensed black nuclei and dark greyish cytoplasm, whereas the majority of those adhering to the peritrophic membrane had appeared normal. On the contrary, in the control group there were no visible abnormal changes and the percentage of ookinetes that developed into oocysts was 65.1% (361.9/556.1).

One of the marked features of the oocysts in the test group was that their DNA level never exceeded that of the oocysts at 48 hours after the blood meal in the control group.

The results indicate that the sporontocidal effect of nitroquine appears to be exerted mainly on the ookinetes, making it difficult for them to pass through the peritrophic membrane, and that nitroquine also interferes with the reproduction of DNA in oocysts.

Editorial note: Nitroquine, 2,4-diamino-6-(3,4-dichlorobenzyl) nitrosamino quinazoline, has the same chemical structure as CI-679:

![Chemical structure of nitroquine]


An antimalarial composite, prepared by mixing piperaquine phosphate and sulfadoxine, was coded Malaria Prophylaxis No. 3 or P-3. Each tablet of P-3 contains 250 mg (150 mg base) of piperaquine phosphate and 50 mg of sulfadoxine. P-3 was used to treat 75 cases of malaria. A total dosage of four or six tablets per patient was administered orally in one to two days. All of these patients were clinically cured within three days, and no relapse occurred during the next 30 days. When prophylactic treatment was given to 7608 non-immune immigrants (each adult receiving four tablets once a month), the incidence of malaria in highly malarious mountain villages dropped from over 10% to 1-2% during the transmission season. The mean time of protection for 87 malaria cases who had received the composite was 19.02±7.38 days, indicating that the actual period of protection effected by P-3 was about 20 days.

Hydroxypiperaquine, 1,3,di-/N-(7-chloro-4-quinolyl)-N-piperazinyi7-propanol-2/, was used to treat 48 cases of falciparum malaria, including 19 cases of chloroquine-resistant subterian malaria. A total dose of 1.5 g was given to adults, while that given to children was decreased according to age. Hydroxypiperaquine phosphate was used as the reference drug in 14 cases. The mean times for fever to subside in the hydroxypiperaquine group and the hydroxypiperaquine phosphate group were 30±3.9 and 21.4±5.8 hours respectively, while the mean parasite clearance times of the corresponding groups were 39±3.9 and 34.6±2.4 hours respectively. The cure rate of falciparum malaria cases taking hydroxypiperaquine was 87%.

Of 19 chloroquine resistant cases, 16 were cured. The mean times for fever to subside and for parasite clearance were 30.9±4.6 and 37.3±2.4 hours respectively.


Hydroxypiperaquine phosphate was used to treat 93 hospitalized cases of acute falciparum malaria, each patient receiving a total dosage of 1.5 g (base) over three consecutive days (0.6 g, 0.6 g, 0.3 g). Chloroquine phosphate was taken as the reference drug. The temperature of all the patients returned to normal within 72 hours after the first dose, the mean time for fever to subside being 28.18±1.62 hours. A majority of 92 patients became negative simultaneously for blood parasites, while in one patient parasitaemia persisted for 96 hours. The mean parasite clearance time was 50.2±4.43 hours. Of 93 patients discharged after seven days of hospitalization, 60 were followed up once weekly for three weeks; recrudescence was seen in only one patient on the 21st day after treatment, the cure rate being 98.3%.

Of 28 patients taking 1.5 g chloroquine, seven showed resistance to the drug, i.e. four of the R II and three of the R III grade as determined by the WHO seven-day test. For 24 patients, the fever subsided within a mean time of 41.96±4.69 hours, while the mean parasite clearance time for 21 patients (the seven resistant patients being excluded) was 67.81±4.65 hours. Of 19 followed-up cases, five had a recrudescence within 21 days after treatment. Of the seven resistant cases, five were clinically cured with subsequent hydroxypiperaquine phosphate treatment.

The difference between hydroxypiperaquine and chloroquine treated cases in either fever subsidence or blood parasite clearance time was statistically significant (p<0.001). The former drug was more effective, with few and mild side effects, such as somnolence, nausea and dizziness and appeared to be also active against chloroquine-resistant falciparum malaria.


The therapeutic effect of hydroxypiperaquine phosphate on chloroquine-resistant malaria in mountainous areas of Hainan Island, Guangdong Province, is reported in this article. The WHO seven-day test showed that of 158 patients who were hospitalized with acute falciparum malaria and who received standard chloroquine regimens, 78 (49.36%) had infections belonging to the sensitive-RI level of resistance category, 12 (7.59%) to the RI level, 45 (28.48%) to the RII level and 23 (14.56%) to the RIII level. Among 66 chloroquine-resistant cases (43 RII and 23 RIII), the 49 adult patients were each given a total oral dosage of 1.5 g (base) of hydroxypiperaquine over three consecutive days, the 17 children being treated with a lower dosage according to age. For the 49 adult cases, the fever subsided within a mean time of 37.18±2.37 hours, and the mean parasite clearance time for the 66 patients was 52.36±2.33 hours.

Of the 64 patients who were followed up, three suffered a recrudescence 21 to 28 days after treatment. The cure rate was therefore 90.22%.
These results suggest that hydroxypiperaquine phosphate is of value in the treatment of chloroquine-resistant falciparum malaria.


The recently developed antimalarial drug, Qinghaosu (artemisinine), is extracted from Artemisia annua L. It is a new type of sesquiterpene lactone with a low toxicity, high efficacy and quick action.

The mutagenic effect of the drug was tested quantitatively, both with and without activation by liver microsomes. The tests were made on five mutant strains of Salmonella typhimurium: TA98, TA100, TA1535, TA1537 and TA1538. The microsomes (S-9) were derived from polychlorinated biphenyl (PCB)-induced male rats' livers. The doses of Qinghaosu used were 0.03, 0.3, 3, 30 and 300 µg per 0.1 ml of dimethylsulfoxide (DMSO) solution.

The results showed no significant increase of reversions induced by the various doses of Qinghaosu in any of the test strains as compared with the spontaneous reversions in the corresponding strains with and without S-9. Since the characteristics of the test strains and the activity of S-9 were well checked, it can be concluded that Qinghaosu was nonmutagenic in this series of experiments.


Artemisinine, a new antimalarial drug discovered by Chinese scientists, and its derivatives were successfully separated by high pressure liquid chromatography both in normal and reversed phases, using silica A and PEAB-ODS-SILX columns, respectively. The packed column, with variable components of solvents as eluents, had been used to study the chromatographic behaviour of each compound, exhibiting a linear relationship between log k' and the logarithm of the concentration of the strong solvents in eluents made up of 2, 2, 4-trimethylpentane and equal volumes of ethanol and methanol.

The quantitative methods for artemisinine and β-methylhydroartemisinine were applied using cholesterol as internal standard and their coefficients of variation were 0.76 and 0.93% respectively.


The chromatographic behaviour of the new antimalarial drugs artemisinine and dihydroartemisinine, its ethers, carboxylic esters, and carbonates, has been studied by using reversed phase high pressure liquid chromatography. These compounds and their epimers can be completely separated on the octadecylsilane bonded stationary phase GYT-G18 under isocratic conditions with methanol-water as eluent; semipreparative separation may also be carried out by an ID 10 mm column packed with the same packing material.

In studying the relationship between the retention and hydrophobicity of the above mentioned compounds, it was observed that the capacity ratio k' increased as the hydrophobicity of the substituent increased, and the correlation of the logarithm of capacity ratio k' with Hansch's hydrophobic substituent parameter was established. Furthermore, the linearity between log k' and chain-length of the alkyl substituent was also obtained.

The effect of the solvent composition on the retention time was studied. The value of log k' decreased linearly as the concentration of methanol in eluent increased.

Artemisinine is a new antimalarial drug which has a marked therapeutic effect on patients infected with the chloroquine-resistant strain of Plasmodium falciparum. In the hope of finding more potent antimalarials, ethers, carboxylic esters and carbonates of dihydroartemisinine were prepared. Most of the 47 new compounds evaluated against the chloroquine-resistant strain of P. berghei in mice were found to be superior to artemisinine itself.

The relationship between chemical structure and antimalarial activity in these derivatives of artemisinine is briefly discussed.

Some compounds tested against Schistosoma japonicum in laboratory animals were also shown to be more active than artemisinine.


Of six crystalline components isolated from the liposoluble fraction of Artemisia annua L., four have been identified as sesquiterpenes, one as flavonol and one as coumarin. Qinghaosu I and III are new sesquiterpenes. Five main constituents, camphene, isoartemisia ketone, l-camphor, β-carophyllene and β-pineene were identified from the volatile oil of this herb.


Methyl dihydroartemisinine (MDHA) is a derivative of artemisinine. Against a chloroquine-resistant strain of Plasmodium berghei in mice its suppressive dose by which 90% of the parasites are eliminated and its minimum effective dose are six and 14 times lower than that of artemisinine respectively. 14C-MDHA was synthesized for studying its in vivo metabolism.

About 12 ml of benzene are added to 426 mg of purified MDHA. After the MDHA is nearly dissolved, 19.8 µl of 14C-methanol and 55.2 µl of methanol are added, being stirred in. Three to four drops of boron fluoride-ether are added to the above mixture which is then left to stand at room temperature for 48 hours. The mixture is filtered through a separatory funnel and an equal volume of saturated solution of sodium acetate is added until neutralization. After separating out the alkali solution, the benzene layer is washed twice with water and the benzene is removed by reduced pressure distillation under 60°C to obtain a mixture of αβ-14C-MDHA. By using silica gel column chromatography, β-14C-MDHA is separated from the mixture.

The radioactivity is detected with a YJS-78 liquid scintillation counter. The values for disintegrations per minute (dpm) for the different tubes are plotted on the ordinate, and the number of tubes on the abscissa. A two peak curve can then be seen, and the high peak corresponds to β-14C-MDHA.


A two-step glutaraldehyde method for the preparation of peroxidase conjugate for the enzyme-linked immunosorbent assay (ELISA) was introduced by Avrameas and Ternynck. The method has been modified and simplified as follows:
(1) Place 5 mg of horseradish peroxidase (HRP) in a test-tube. Add 0.4 ml of 0.05 M carbonate buffer pH 9.6 to dissolve the enzyme, then add 0.1 ml of 25% glutaraldehyde ($A235/A280 = 10$), mix well and incubate at 37°C for two hours. Then add 0.1 ml of 22% NaCl solution. Cool the solution. Introduce 2.4 ml of cold absolute ethyl alcohol (approximately 5°C) into the test-tube, mix thoroughly. Centrifuge at 1000 rpm for 10-15 minutes and remove the supernatant. Wash the precipitate with 80% cold ethanol, centrifuge as above. Pour out alcohol as much as possible. Add 0.5 ml of 0.05 M carbonate buffer pH 9.6 to dissolve the precipitate. Mix it with 0.5 ml of antibody solution containing 5-10 mg IgG. Keep in refrigerator over night. The preparation is ready for use.

(2) Another method is a modification of the periodate method developed by Nakane and Kawaoi, using formaldehyde (final concentration 0.6%) or glutaraldehyde (final concentration 5%) instead of 1-fluoro-2, 4-dinitrobenzene, to avoid the production of hydrogen fluoride (HF), which is an inhibitor of HRP. The carbohydrate portion of the enzyme is oxidized by 0.03 M periodate solution (final concentration) at 0-5°C for 30 minutes. The oxidized enzyme is precipitated by cold absolute ethanol. The precipitate is washed with 80% alcohol, dissolved in 0.05 M carbonate buffer, and then coupled with antibody (10-15 mg).

References

Avrameas, S. & Ternynck, T. (1971) Immunochemistry, 8, 1175


Two different antigens from Plasmodium knowlesi were used to immunize five rhesus monkeys each. The specific antibody was detected by the indirect fluorescent antibody technique, using a thin blood smear of P. knowlesi as antigen and fluo-fluorescein isothiocyanate-sheep anti-human globulin as fluorescein-antiglobulin. Another five rhesus monkeys receiving normal saline intramuscularly were used as control. Nineteen days after the first immunizing injection, the antibody titre had risen from 1:10-40 to 1:160, while that of the control monkeys remained at the level of 1:10-40. On the ninth day of the third immunizing injection, 11x10^3 infected red blood cells were injected for challenge. Five days after challenge, the antibody titre rose to 1:640-1280, while that of the controls was below 1:40. In the five surviving monkeys, the antibody titre began to drop on the 61st day after the first injection, and had returned to the level at which it was before immunization after 161 days. The antibody titre could not reflect the protective immunity, as both the monkeys which survived and those which succumbed had the same antibody level.


During the period 27 October - 10 November 1978, railway builders and their families, students and children who came to the construction area in Henan at the beginning of that year were investigated by the indirect fluorescent antibody (IFA) test with antigen prepared from Plasmodium cynomolgi. The construction area was endemic for P. vivax during the June-September transmission season.

As a result, 339 out of 2065 specimens of blood dried on filter paper were found to be IFA positive (i.e. 16.4%), the total geometric mean reciprocal titre (GMRT) being 18.71 and the GMRT of the positive sera being 45.38. There was no significant difference between the different age groups or between males and females. However, the IFA positive rates in various localities were consistent with the malaria incidence in these localities. It would seem that the antibody titres could reflect the malaria transmission level, and the positive GMRT in the 107 cases of symptomatic malaria was higher than that in 232 asymptomatic cases, being 80.98 and 37.71 respectively.