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# In Vitro Activities of Pyronaridine, Alone and in Combination with Other Antimalarial Drugs, against *Plasmodium falciparum*

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**The in vitro activities of pyronaridine, alone and in combination with established antimalarial drugs, were assessed by isotopic microtest. Pyronaridine was highly active against all Cameroonian isolates. A positive correlation was observed between the response to pyronaridine and that to chloroquine. Drug combination studies showed synergy between pyronaridine and primaquine, additive effects with 4-aminoquinolines, and weak antagonism with dihydroartemisinin, antifolates, or amino alcohols.**

Pyronaridine, an acridine-type (benzonaphthyridine) Mannich base synthesized in China, has been shown to be well tolerated and highly effective in treating malaria-infected patients in chloroquine resistance regions (4, 10, 15, 16). The World Health Organization plans to complete the preclinical and clinical trials with the aim of registering pyronaridine in areas of endemicity and replacing chloroquine with pyronaridine for the first-line treatment of malaria in Africa. The development of pyronaridine raises the important question of the expected life span of the drug in chloroquine resistance zones. The answer to this question depends partly on two factors. First, the possible existence of cross-resistance between chloroquine and pyronaridine may rapidly compromise the efficacy of pyronaridine in areas where chloroquine resistance is widespread. Second, the life span of pyronaridine will also depend on whether it will be used in monotherapy or in combination with another antimalarial drug (19).

Previous in vitro and in vivo studies have drawn contradictory conclusions on the relationship between the blood schizontocidal activity of pyronaridine and that of chloroquine (1, 5, 11, 13, 18). We have evaluated the potential for in vitro cross-resistance between pyronaridine and various antimalarial drugs, in particular chloroquine, with a large number of clinical isolates obtained in Yaoundé, Cameroon. We have also studied the in vitro interaction between pyronaridine and other antimalarial drugs with the aim of identifying a suitable drug that may be used in combination with pyronaridine.

As part of our clinical studies (15, 16), fresh clinical isolates of *Plasmodium falciparum* were obtained before treatment from 183 symptomatic Cameroonian patients attending the Nlongkak Catholic missionary dispensary in Yaoundé between 1994 and 1998. This study was approved by the Cameroonian national ethics committee and the Cameroonian Ministry of Public Health. For drug interaction studies, the chloroquine-resistant W2/Indochina clone was maintained in continuous culture. The clone was synchronized by treating the infected erythrocytes with 5% D-sorbitol (8). The isotopic microtest developed by Desjardins et al. was used in this study (6). The sources of antimalarial drugs and the preparation of drug-coated assay plates and suspension of infected erythrocytes were described in our previous study (14). The 50% inhibitory concentrations ($IC_{50}$) were determined by nonlinear regression analysis. Pyronaridine was combined with different antimalarial drugs to determine the type of interaction between drugs (2). Starting concentrations corresponding to 10 times the $IC_{50}$ of the test compounds alone were mixed at three different ratios (1:3, 1:1, and 3:1 [vol/vol]), and twofold dilutions were distributed in 96-well tissue culture plates in triplicate. The assays were performed with the W2 clone at an initial parasitemia of 0.6%. Each drug combination was tested three times. Results were expressed as the mean sums of the fractional inhibitory concentrations (FIC), defined as ($IC_{50}$ of drug A in mixture/$IC_{50}$ of drug A alone) + ($IC_{50}$ of drug B in mixture/$IC_{50}$ of drug B alone) for each fixed concentration (2, 3). Three types of drug interaction were defined as follows: additive, sum of FIC = 1; synergistic, sum of FIC < 1; and antagonistic, sum of FIC > 1 (2).

Of the 183 isolates, 82 (44.8%) and 101 (55.2%) were sensitive and resistant to chloroquine, respectively (Table 1). Pyronaridine was highly active in vitro against all Cameroonian isolates. The overall geometric mean $IC_{50}$ of pyronaridine was 3.79 nmol/liter (95% confidence intervals, 3.47 to 4.13 nmol/liter; range, 0.92 to 18.3 nmol/liter; $n = 183$). There was no statistical difference between the mean $IC_{50}$ of pyronaridine for the chloroquine-sensitive isolates and that for the chloroquine-resistant isolates (unpaired $t$ test; $P > 0.05$). The responses of pyronaridine and chloroquine were positively correlated, but the coefficient of correlation calculated by a linear regression analysis of logarithmic $IC_{50}$ ($r = 0.159$, $P < 0.05$) was low. Similar relationships were found between pyronaridine and quinine ($r = 0.392$, $P < 0.05$) or halofantrine ($r = 0.317$; $P < 0.05$). The $IC_{50}$ of pyronaridine and mefloquine ($r = 0.525$; $P < 0.05$) or artemether ($r = 0.556$; $P < 0.05$) were moderately correlated. There was no correlation ($P > 0.05$) between pyronaridine and monodesethylamodiaquine ($r = 0.132$) or antifolate drugs ($r = 0.029$). A high correlation was found between chloroquine and monodesethylamodiaquine ($r = 0.853$; $P < 0.05$) and between pyrimethamine and cycloguanil ($r = 0.977$; $P < 0.05$). Drug interaction was studied with the chloroquine-resistant W2/Indochina clone (Table 2). The combinations of pyronaridine and dihydroartemisinin, antifolate drugs (pyrimethamine and cycloguanil), or amino

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TABLE 1. In vitro activity of pyronaridine and other antimalarial drugs against the chloroquine-sensitive and the chloroquine-resistant Cameroonian isolates of *P. falciparum*

| Drug | Value for isolate: | | | | | |
|---|---|---|---|---|---|---|
| | Chloroquine sensitive | | | Chloroquine resistant | | |
| | *n* | Mean[a] | 95% confidence intervals | *n* | Mean[a] | 95% confidence intervals |
| Chloroquine | 82 | 33.2 | 29.7–37.2 | 101 | 283 | 256–313 |
| Monodesethyl-amodiaquine | 24 | 14.7 | 11.6–18.6 | 48 | 49.2 | 42.3–57.3 |
| Quinine | 23 | 100 | 75.5–133 | 46 | 194 | 158–237 |
| Mefloquine | 20 | 7.72 | 5.41–11.0 | 32 | 8.12 | 6.18–10.7 |
| Halofantrine | 22 | 1.53 | 1.16–2.01 | 45 | 1.17 | 0.98–1.38 |
| Artemether | 22 | 2.50 | 1.90–3.29 | 43 | 1.54 | 1.20–1.99 |
| Pyrimethamine | 10 | 34.8 | 6.88–176 | 29 | 381 | 118–1,230 |
| Cycloguanil | 10 | 15.4 | 4.57–51.9 | 29 | 91.8 | 37.4–225 |
| Pyronaridine | 82 | 3.58 | 3.16–4.06 | 101 | 3.97 | 3.52–4.48 |

[a] Geometric mean $IC_{50}$ (nanomoles per liter).

alcohols (quinine, mefloquine, and halofantrine) were antagonistic. The combinations of pyronaridine and 4-aminoquinolines (chloroquine and monodesethylamodiaquine) were additive. The pyronaridine-primaquine combination was synergistic.

Pyronaridine has been shown to be highly active in vitro against field isolates and laboratory-adapted strains originating from various geographic regions, with $IC_{50}$ below 50 nmol/liter (1, 5, 7, 13). Our results further demonstrate the potent in vitro activity of pyronaridine against the chloroquine-sensitive and the chloroquine-resistant clinical isolates of *P. falciparum* in Yaoundé, Cameroon. Of 183 isolates, 66 were obtained from patients who were treated with oral pyronaridine (15, 16). Thirty-four of 66 isolates (52%) were resistant in vitro to chloroquine. The pyronaridine $IC_{50}$ for the isolates from these patients, all of whom cleared parasitemia during the 14-day follow-up, ranged from 1.3 to 14.7 nmol/liter.

The responses of pyronaridine and chloroquine were slightly correlated in our in vitro study. Similar results were reported by Pradines et al. for African isolates originating from Senegal, West Africa (13). Our in vitro results suggest that high coefficients of correlation are generally observed between antimalarial drugs which share chemical features (mefloquine and halofantrine; chloroquine and monodesethylamodiaquine) and/or inhibit the same molecular target (pyrimethamine and cycloguanil). Clinical studies have shown that pyronaridine is effective in obtaining parasite clearance on day 14 in African and Thai patients infected with chloroquine-resistant *P. falciparum* (10, 15, 16). Thus, although the correlation of in vitro and in vivo results is limited, there is circumstantial evidence that the slight in vitro correlation between pyronaridine and chloroquine is overcome or masked in vivo, even in areas where chloroquine resistance has attained a high level. Further clinical trials of pyronaridine are needed to confirm its efficacy in chloroquine resistance zones.

Despite the encouraging results of the clinical studies, experimental studies have demonstrated that resistance to pyronaridine can be rapidly induced (11, 12, 17). Furthermore, the monitoring of in vitro drug sensitivity has shown that 13 of 156 (8%) clinical isolates in southern China were resistant to pyronaridine (9). Yang et al. (20) have also reported that the in vitro sensitivity of *P. falciparum* isolates in southern China decreased between 1988 and 1995 and, in parallel, the recrudescence rate following pyronaridine treatment increased from 5 of 33 cases (15%) in 1984-1985 to 9 of 24 cases (38%) in 1995. These recent findings imply that pyronaridine-resistant *P. falciparum* may already have emerged in China.

Drug combination is one of the effective means to counter drug resistance in antimalarial chemotherapy (19). The ideal drug partner of pyronaridine should exert a synergistic or additive schizontocidal action. In the present study, additive interaction was observed between pyronaridine and 4-aminoquinolines. Because resistance to 4-aminoquinolines is widespread in certain areas of endemicity, a combination of these drugs with pyronaridine may not remain useful over a long period. Antagonistic interactions were obtained with pyronaridine and antifolate drugs, amino alcohols, or dihydroartemisinin. However, FIC at different fixed combinations did not surpass, or were only slightly superior to, the $IC_{50}$ of the test compounds alone normalized to 1 isobolar U, which signifies that there was only a weak antagonistic effect (3). A strong antagonism, by definition, refers to the loss of schizontocidal effect when the drugs are used in combination, requiring higher concentrations of the drugs to produce the same effect as the drugs alone (2). Since both artemisinin derivatives and pyronaridine are effective at present, the artemisinin-pyronaridine combination may be one of the rational choices to protect both drugs from the selection of resistant *P. falciparum* strains. In the rodent malaria model, the pyronaridine-artemisinin combination was additive against the chloroquine-resistant strain and synergistic against the artemisinin- or pyronaridine-resistant strains of *Plasmodium yoelii* (12). Artemisinin derivatives are rapidly eliminated and reduce the parasite load considerably within a single life cycle of the parasites, and residual parasites may be eliminated by a second drug with a minimal risk of selecting mutant, resistant parasite populations (19). The pyronaridine-primaquine combination yielded a synergistic interaction in vitro, but in view of its side effects, long duration of therapy, and inadequate blood schizontocidal action, in vivo studies of this combination do not seem to be justified. Further studies on the combination of pyronaridine and artemisinin derivatives or other 8-aminoquinolines that are more active and less toxic than primaquine are needed to assure the safety and efficacy of these combinations.

TABLE 2. Drug interaction between pyronaridine and other antimalarial drugs

| Drug coadministered with pyronaridine[a] | FIC[b] | Interaction |
|---|---|---|
| Chloroquine | 1.09 | Additivity |
| Monodesethylamodiaquine | 0.99 | Additivity |
| Primaquine | 0.65 | Synergy |
| Quinine | 1.55 | Antagonism |
| Mefloquine | 1.54 | Antagonism |
| Halofantrine | 1.16 | Antagonism |
| Dihydroartemisinin | 1.33 | Antagonism |
| Pyrimethamine | 1.10 | Antagonism |
| Cycloguanil | 1.37 | Antagonism |

[a] The sensitivity profile (mean $IC_{50}$ for three to five independent experiments) of the W2/Indochina clone was as follows (values in nanomoles per liter): pyronaridine, 22.1; chloroquine, 445; monodesethylamodiaquine, 96.6; quinine, 510; mefloquine, 18.2; halofantrine, 2.70; dihydroartemisinin, 3.80; pyrimethamine, 18,300; cycloguanil, 1,530; and primaquine, 1,650.

[b] FIC of 1:1 fixed combination. Additivity, FIC = 1; synergy, FIC <1; antagonism, FIC > 1. Although the FIC of pyronaridine-halofantrine and pyronaridine-pyrimethamine were close to additivity at a 1:1 fixed combination, higher FIC (antagonism) were obtained at different fixed combinations.

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