In vitro susceptibility to a new antimalarial organometallic analogue, ferroquine, of *Plasmodium falciparum* isolates from the Haut-Ogooué region of Gabon

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Objectives: To assess the activity of a new organometallic chloroquine analogue, ferroquine, against numerous *Plasmodium falciparum* isolates from Gabon.

Methods: The in vitro susceptibility of 116 *P. falciparum* isolates to chloroquine and ferroquine was assessed using the isotopic microtest. All isolates were from outpatients in the Franceville and Bakoumba medical centres in the province of Haut-Ogooué, south-east Gabon.

Results: The in vitro resistance to chloroquine was 51.8% in Franceville and 96.7% in Bakoumba. The IC₅₀ geometric mean (95% CI) of ferroquine against isolates in Franceville was 16.0 (14.4–17.8) nM, with individual values ranging from 1.0 to 47.0 nM; in Bakoumba it was 27.9 (23.4–33.2) nM, with individual values ranging from 1.0 to 62.0 nM. Compared with chloroquine, ferroquine was 5.3 times more active on isolates susceptible to chloroquine, and 13.3 times more active on isolates resistant to chloroquine. A weak positive correlation was observed between responses of these two drugs, but too low to demonstrate cross-resistance.

Conclusions: Ferroquine may be useful as an alternative drug for treating chloroquine-resistant malaria.

Keywords: chloroquine, ferrocene, malaria

Introduction

After a period of stabilization, morbidity and mortality due to malaria are increasing again. Almost half of humanity lives in areas where malaria is transmitted. Each year, this parasite kills 1.5–2.7 million people, including 1 million children under 5 years of age in Africa alone. *Plasmodium falciparum* is the most frequent species and is responsible for the most deadly human malaria.¹

During the last two decades, the most disturbing phenomenon has been the increase in drug resistance and its extension to almost all molecules available in malaria chemotherapy. Resistance to chloroquine, the oldest and most used antimalarial drug because of its efficiency, tolerance, low cost and availability, has spread to all endemic regions. *P. falciparum* has become gradually resistant to all other antimalarial compounds, including quinine, mefloquine and halofantrine. The general spread of drug resistance or multi-resistance limits the efficiency of malaria treatment. Facing this situation, the availability of new antimalarial drugs becomes urgent. Ferroquine is an organometallic analogue synthesized from a new strategy consisting of the association of one ferrocene molecule with chloroquine (Figure 1).² The derived molecule is active against chloroquine-resistant *P. falciparum*...
strains in vitro and in vivo. The present study aims to confirm the efficiency of ferroquine against numerous *P. falciparum* isolates from the Haut-Ogooué area of Gabon.

**Materials and methods**

**Parasites**

Patients attending Franceville or Bakoumba hospitals were screened for malaria parasites. Giemsa-stained thin and thick blood smears were examined. Blood samples presenting with a pure *P. falciparum* parasite density >0.02% were included in the study. Venous blood samples were collected after patients’ informed consent. If parasitaemia exceeded 1%, uninfected erythrocytes were added to adjust parasitaemia to 1%. This study was approved by the Human Ethics Committee of the Centre International de Recherches Médicales de Franceville.

**In vitro assay**

Ferroquine dichlorhydrate (FQ: 7-chloro-4-[(2-N,N′dimethylaminomethyl) ferrocenylmethylamino]quinoline), synthesized as described, was obtained from Laboratoires Pierre Fabre (Labège Innopole, France). Chloroquine diphosphate (CQ) was from Sigma Chemicals (St Louis, MO, USA). Stock solutions of chloroquine and ferroquine were prepared in ethanol, with final ethanol concentrations not exceeding 0.05%. All concentrations, including drug-free controls, were distributed in triplicate in 96-well tissue culture plates. *In vitro* testing was carried out as described, using an isotopic micro-drug susceptibility assay. The concentration–response data were interpreted by linear regression analysis of the concentration logarithm against growth inhibition. The IC$_{50}$ (50% inhibitory concentration) was defined as the drug concentration corresponding to 50% of the uptake of [3H]hypoxanthine in the drug-free control wells. The threshold IC$_{50}$ value for resistance to chloroquine was 100 nM. Data were expressed as geometric mean IC$_{50}$ values ± S.E. Relations between the IC$_{50}$ values of the two drugs for a given isolate were computed by linear regression analysis using Statview software (SAS Institute Inc., Cary, NC, USA).

**Discussion**

The extension of *P. falciparum* drug resistance to all endemic areas has discouraged any attempt to eradicate malaria. Hitherto, the only available strategy was to reduce malaria-related mortality and morbidity, using chemoprophylaxis and chemotherapy. In Gabon, *P. falciparum* chloroquine resistance was first detected in the 1980s, and spread rapidly, reaching 90–100% *in vitro*. *P. falciparum* resistance to quinine, sulphamamide–pyrimethamine association, mefloquine, halofantrine and artemisinine and its derivatives is also reported in Gabon. As drugs of natural origin are lacking, part of the research is turning towards the synthesis of new molecules active against drug-resistant parasites. Ferroquine synthesis is based on the association of one standard antimalarial molecule, chloroquine, with an organometallic complex, ferrocene. Ferrocene is not toxic to humans and contains an iron atom very attractive to *Plasmodium* parasites. We tested the *in vitro* sensitivity of 116 *P. falciparum* isolates to ferroquine in Haut-Ogooué, south-east Gabon. Ferroquine activity was higher than that of chloroquine, with a mean IC$_{50}$ value of 22.2 nM; all IC$_{50}$ being <62 nM. Prevalence of chloroquine resistance was moderate in Franceville (52%), but very high (97%) in Bakoumba. No matter what the chloroquine resistance level,
rapidly. The storage of non-protonated chloroquine in the resistant parasites show chloroquine incorporation kinetics the haemoglobin degradation by the parasite. Chloroquine inhibits the toxic free haem polymerization derived from concentration in the parasite digestive vacuole, chloroquine correlated, the intensity of this relationship was limited. Such quinine and therefore retain it in the digestive vacuole, thus pre-

pH, or proton pumps could have a weaker affinity for ferro-

form inside the digestive vacuole albeit at a slightly alkaline exit in resistant strains. Ferroquine could keep its protonated to the fact that deficient proton pumps favour chloroquine resistance. Even though the mode of action of ferrocene is not well understood, ferroquine restores chloroquine activity totally on chloroquine-resistant parasites. As an alternative drug to chloroquine, ferroquine may find a place of choice in the armamentarium for treating chloroquine-resistant malaria.

Ferroquine antimalarial activity in Gabon

![Figure 2. In vitro activity (IC50) of chloroquine and ferroquine against 116 P. falciparum isolates from Haut Ogooué province, Gabon.](image)

ferroquine was five to 15 times more efficient than chloro-

quine. In our study, ferroquine was less active than against P. falciparum parasites from Senegal,5 probably due to the difference in chloroquine sensitivity between the two areas. In Libreville, Gabon, where most P. falciparum isolates are resistant to chloroquine, ferroquine activity was also lower than in Senegal.4 Ferroquine activity was similarly high in vitro against laboratory lines of P. falciparum,3 and in vivo against clones of Plasmodium berghei and Plasmodium vinckei.2,7

Although the activities of chloroquine and ferroquine were correlated, the intensity of this relationship was limited. Such correlation of activities was previously observed,4,5 also with limited value of the coefficient of determination (r2). Such values of r2 suggest common features in drug intake and/or mode of action or resistance rather than true in vitro cross-resistance or common mechanisms of action. To involve the same mechanism of action for two compounds, the coefficient of determination must be higher. The high activity of ferroquine on chloroquine-resistant isolates suggests either that ferroquine and chloroquine have different modes of action, or that ferroquine reverses chloroquine resistance. The better activity of ferroquine than chloroquine (five to seven times) on chloroquine-susceptible isolates suggests that the organometallic complex exerts its own antimalarial activity, and does not act only by potentializing the action of chloroquine, contrary to what has previously been stated.3

The best accepted hypothesis suggests that, because of its concentration in the parasite digestive vacuole, chloroquine inhibits the toxic free haem polymerization derived from the haemoglobin degradation by the parasite. Chloroquine-resistant parasites show chloroquine incorporation kinetics identical to susceptible strains, but release chloroquine more rapidly.8 The storage of non-protonated chloroquine in the digestive vacuole cannot be maintained because of the perme-

ability of the vacuole membrane, due to the lack of acidity or to the fact that deficient proton pumps favour chloroquine exit in resistant strains. Ferroquine could keep its protonated form inside the digestive vacuole albeit at a slightly alkaline pH, or proton pumps could have a weaker affinity for ferro-

quine and therefore retain it in the digestive vacuole, thus pre-

paring its antimalarial action against chloroquine-resistant strains. The molecular structure of ferroquine is very important in resistance mechanisms. In strategies looking for more active organometallic antimalarial drugs, the chloroquine molecule has been modified, but none of the derived analogues showed an antimalarial activity higher than that of chloroquine or ferroquine. Analogues in which the ferrocenyl-methyl group was attached to the nitrogen atom inside the quinoline nucleus,7 or in which the ferrocene was not linked to chloroquine by a covalent bond,3 had reduced activity. In contrast, substitution of an ethyl group by a ferrocenyl-methyl on the terminal nitrogen of the chloroquine molecule side chain gave increased antimalarial activity. The substituted ferrocene seems to bring important changes in molecule proper-

ties, such as the increase in solubility and lyophilization.7 The attachment of two methyl radicals on the terminal nitrogen of the molecule side chain yields a more active product than ferrocenic diethylated analogues.3 The position of ferrocene in the molecule therefore plays an important role, providing antimalarial activity only when placed inside the side chain. Ferrocene needs to be linked covalently to chloro-

quione to yield a significant antimalarial effect. The ferrocene activity seems to be closely related to the chemical structure of these ferrocenic analogues.

Transition metal complexes are useful in medicine and biology. Platinum complexes have been successfully used as anti-tumour agents. In malaria, iron complexes using ferriox-

amine hydroxyamate deprive the parasite of iron, an essential metal for its survival. The gold–chloroquine complex is active in vitro and in vivo on P. berghei, the gold moiety pro-

viding an important increase in chloroquine efficiency.9,10 P. falciparum malaria is a major obstacle to the develop-

ment of individuals, communities and nations. New active compounds will help to deal with severe malaria or multidrug resistance. Nevertheless, only molecules with original modes of action may stop the present frightening spread of drug resistance. Even though the mode of action of ferrocene is not well understood, ferroquine restores chloroquine activity totally on chloroquine-resistant parasites. As an alternative drug to chloroquine, ferroquine may find a place of choice in the armamentarium for treating chloroquine-resistant malaria.

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