

ISRADIPINE – A CALCIUM CHANNEL BLOCKER – DOES NOT POTENTIATE CHLOROQUINE ANTIPLASMODIAL ACTIVITY AGAINST *PLASMODIUM FALCIPARUM*

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Summary:

Culturing fresh clinical isolates of *P. falciparum* and using the isotopic method, we tested separately chloroquine and isradipine – a calcium channel blocker –, and also the combination isradipine plus chloroquine. Tested wild isolates were chloroquine-sensitive. With regard to the combination isradipine/chloroquine, the isobolograms obtained indicate that isradipine antagonises chloroquine antiplasmodial activity. Taking into account these findings, we discuss the issues related to the calcium channel blocker molecules.

KEY WORDS : chloroquine, isradipine, *Plasmodium falciparum*, Madagascar.

Résumé : L'ISRADIPINE – UN INHIBITEUR DE CANAUX CALCIQUES – NE POTENTIALISE PAS L'ACTIVITÉ ANTIPLASMODIALE DE LA CHLOROQUINE SUR *PLASMODIUM FALCIPARUM*

Des isolats de *P. falciparum* fraîchement prélevés de malades ont été testés pour évaluer leurs réponses *in vitro* à la chloroquine, à l'isradipine (un inhibiteur de canaux calciques), et à la combinaison isradipine/chloroquine. Les tests de chimiosensibilité ont été réalisés avec la méthode isotopique. Ces isolats sauvages étaient sensibles à la chloroquine. Quant à la combinaison isradipine/chloroquine, les isobogrammes obtenus indiquent un antagonisme entre les deux molécules. Tenant compte de ces résultats, nous soulevons dans cet article l'importance de la compréhension du métabolisme calcique des parasites du paludisme ; et l'énigme de la réversion de la chloroquinorésistance par certaines molécules inhibitrices des canaux calciques.

MOTS CLÉS : chloroquine, isradipine, *Plasmodium falciparum*, Madagascar.

Martin *et al.* (1987) demonstrated that the calcium channel blocker verapamil, a phenylalkylamine, reverses chloroquine resistance in *Plasmodium falciparum*. Subsequent studies were then carried out to assess the nature of the interaction between antimalarial molecules and different calcium channel blockers in *P. falciparum* in order to have new means of controlling drug resistant parasites (Scheibel *et al.*, 1987; Kyle *et al.*, 1990). Most of the studies of the interaction between channel blockers and antimalarial drugs were performed with *P. falciparum* clones maintained in continuous cultures. In this preliminary study, *in vitro* testing was carried out with Malagasy (Madagascar) fresh isolates of *P. falciparum* exposed to dihydropyridine channel blocker isradipine (Fig. 1), a calcium channel blocker which is frequently prescribed to treat essential hypertension (Persson *et al.*, 1989), and to chloroquine.

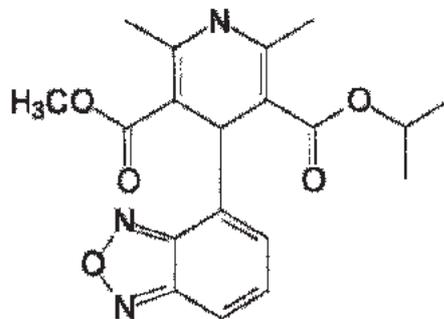


Fig. 1. – Chemical structure of isradipine.

MATERIAL AND METHODS

IN VITRO *PLASMODIUM FALCIPARUM* CHEMOSENSITIVITY TESTS

Clinical *P. falciparum* isolates were collected from consenting patients in Saharevo. Samples were transported to the Malaria Research Unit at the "Institut Pasteur de Madagascar" where the isotopic *in vitro* chemosensitivity tests were performed. Chloroquine diphosphate (Sigma Chemicals) was tested as described elsewhere (Randrianariveლოსია *et al.*, 2002). Isradipine (Icaz® LP, Laboratoires Sandoz, France) test

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concentrations ranged from 2 to 200 µM. The effect of isradipine on the activities of the antimalarials was determined by combination test (Martin *et al.*, 1987). The chloroquine-resistant strain *P. falciparum* FCM29 maintained in continuous culture was also tested immediately after synchronization using the sorbitol-based method (Lambros & Vanderberg, 1979).

2.5 and 6 µM of isradipine were separately combined with chloroquine. Isobolograms were constructed by plotting a pair of fractional IC50s for each combination of isradipine and the antimalarial drugs. Antimalarial drug fractional IC50s were calculated by dividing the IC50 of the drug combined with isradipine by the IC50 of the drug alone, and these data were plotted on the horizontal axis. The corresponding isradipine fractional IC50 was calculated by dividing each fixed concentration by the IC50 of isradipine alone. These data were plotted on the vertical axis. Only combined isradipine concentrations less than isradipine IC50 (tested alone) were used in the final analysis. An isobologram close to the diagonal indicates an additive effect. Curves significantly above or below the diagonal indicate antagonistic or synergistic effects, respectively (Berenbaum, 1978; Martin *et al.*, 1987).

RESULTS AND DISCUSSION

IC50 values are reported in the table. Chloroquine plus isradipine was successfully tested on three fresh wild isolates of *P. falciparum* and also on *P. falciparum* FCM29 strain. In the three isolates, isobolograms shape indicated the antagonistic effect of isradipine on chloroquine antiplasmodial activity, while in FCM29 the isobologram almost indicates additive effects (Fig. 2; Table I).

Martiney *et al.* (1995) reported that in short time incubation, verapamil was found to increase chloroquine accumulation in erythrocytes infected with both chloroquine-sensitive and -resistant parasites, but only to affect the chloroquine susceptibility of the latter. Since

	Isradipine IC50 in µM	Chloroquine IC50 in nM
<i>P. falciparum</i> wild isolates ^a		
96065	11.4	33.9
96067	12.9	51.1
96068	15.3	64.5
<i>P. falciparum</i> FCM29 strain ^b	23.2 ± 5.8 ^c	316 ± 21.6 ^c

a: tested once; b: tested three times; c: 2 standard deviation.

Table I. – *In vitro* response of three chloroquine-sensitive *P. falciparum* isolates and of chloroquine-resistant *P. falciparum* FCM29 strain to isradipine and chloroquine *in vitro*.

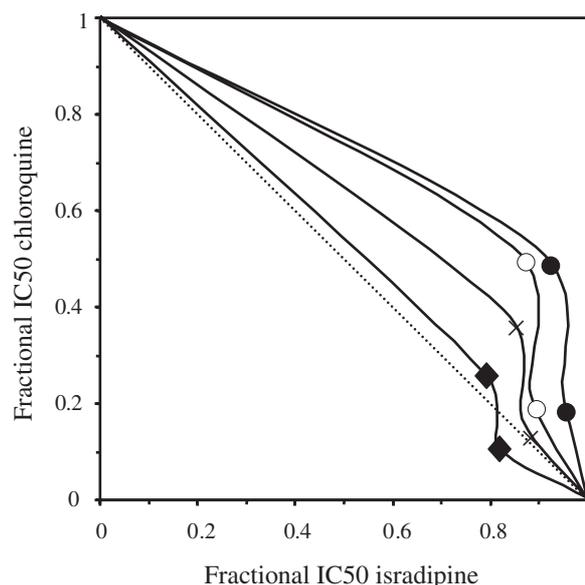


Fig. 2. – Isobolograms of *in vitro* drug interaction between isradipine and chloroquine against the chloroquine-resistant *P. falciparum* FCM29 strain (◆) and against three chloroquine-sensitive wild isolates of *P. falciparum* coded 96065 (×); 96067 (○) and 96068 (●) from Madagascar.

tested isolates were chloroquine-sensitive (and did not harbour mutant *pfcr*t parasites, PCR/RFLP data not shown), it should not be surprising that isradipine does not potentiate chloroquine activity. But this does not explain the antagonism between isradipine and chloroquine.

Persson *et al.* (1989) reported that the mean plasma concentration of isradipine three hours after tablet intake was 3.14 ng/ml in 15 patients treated for essential hypertension. By comparison with this average therapeutic plasmatic concentration, the combined isradipine concentrations in our *in vitro* study were over 250 times higher. That makes questionable the relevance of these *in vitro* findings with regard to the possible *in vivo* drug interaction with chloroquine in a patient taking isradipine.

Thus, our preliminary results demonstrate that on the basis of isobologram, isradipine antagonises chloroquine antiplasmodial activity in wild chloroquine-sensitive *P. falciparum*, however only at very high concentrations. Even in chloroquine-resistant strain *P. falciparum* FCM29, isradipine does not potentiate chloroquine activity.

Isradipine belongs to the dihydropyridine chemical family as does amlodipine, while verapamil is a phenylalkylamine. Still our results demonstrate that the calcium channel blocking properties are in no way correlated with systematic resistance reversal as already pointed out by Basco & Le Bras (1991) when they showed that the reversal of chloroquine resistance by the enantiomers of amlodipine is independent of calcium metabolism of malaria parasites. The mechanism of resistance reversal

by calcium channel blockers still remains unclear. But Mercereau-Puijalon & Fandeur (2003) reported the intriguing possibility that both mefloquine and artemisinins could interfere with calcium homeostasis. A better understanding of the calcium homeostasis in malaria parasites would be an advance for malaria therapy.

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