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Antimalarial activity of optical isomers of quinacrine dihydrochloride against chloroquine-sensitive and -resistant *Plasmodium falciparum in vitro*

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While chloroquine has for many years been the mainstay of malaria prophylaxis and treatment, the extensive spread of resistance to this drug in *Plasmodium falciparum* is threatening global malaria control. Before the introduction of chloroquine, quinacrine (mepacrine, atebrin) fulfilled prophylactic and treatment functions, but did show a higher proportion of toxic effects (for example, see Ref. 1) and its use has decreased.

Chloroquine and quinacrine are synthetic drugs which each possess a single asymmetric (chiral) centre in the diamino side-chain. Commercial preparations are racemic mixtures of the (+) and (−) enantiomers. Fink *et al.* [2] and Haberkorn *et al.* [3] in studies on chloroquine against *P. vinckei* and *P. berghei* in mice found that the (+) enantiomer was more active than the (±) or (−) forms, but that this difference disappeared against chloroquine-resistant strains [3]. Fu *et al.* [4] reported that the enantiomers and the racemate were equally active *in vitro* against the chloroquine-sensitive F32 strain of *P. falciparum* but the (−) enantiomer was less active against the chloroquine-resistant K1 strain, as shown in their figure 2 [4].

A number of reports suggest that the metabolism of both chloroquine and quinacrine may be stereoselective. Thus, Titus *et al.* [5] found that the metabolite desethylchloroquine excreted by man after treatment with racemic chloroquine was optically active with $[\alpha]_D^{25}$ + 145°. It was recently reported by Ofori-Adjei *et al.* [6] that stereoselective renal clearance of the chloroquine enantiomers occurred and [7] that the enantiomers differed in their binding to plasma, to albumin, and to α_1 acid glycoprotein.

In the case of quinacrine similar evidence for stereoselective disposition was uncovered by Hammick and Chambers [8] who found that quinacrine recovered from human urine after treatment with the racemic drug exhibited a rotation $[\alpha]_D$− 368°, corresponding to one enantiomeric form [9].

Since the enantiomers of quinacrine were recently obtained by synthesis [10, 11] and have not been examined previously for differential antimalarial activity either *in vitro* or *in vivo*, the present study was performed to determine the *in vitro* antimalarial activity of all three isomers of quinacrine against both chloroquine-sensitive and -resistant *P. falciparum*. At the same time, a parallel series of experiments was carried out using the corresponding isomers of chloroquine [10].

Materials and Methods

Test substances. The optical isomers of chloroquine were prepared by a synthesis starting from D- and L-glutamic acid [10] and were of >90% optical purity. The enantiomers of chloroquine diphosphate had m.p. 202° and $[\alpha]_D^{25}$ ± 69° (c = 2,H_2O). The enantiomers of quinacrine were obtained by an analogous method using the same optically-active side-chain [10] which gave material of similar optical purity. The enantiomeric quinacrine dihydrochlorides had m.p. 259–261° and $[\alpha]_D^{25}$ ± 351° (c = 2,H_2O).

Malaria. The strains of *P. falciparum* used were continuously cultured in our laboratories by standard techniques [12, 13]. The Honduras CDC1 strain [14] is chloroquine-sensitive and pyrimethamine-resistant, whilst the Thailand K1 strain is resistant to chloroquine, pyrimethamine and sulfadoxine [15].

Drug tests. The activity of the drugs was measured in 96-well microplates by the [^{3}H]hypoxanthine incorporation technique [16, 17]. Activities were also confirmed independently by microscopy. Inhibition/log drug concentration curves were analysed by regression analysis of the linear portion of the curve. IC_{50} values were calculated, together with their 95% confidence limits, and are shown in Table 1.

Results and Discussion

For chloroquine, the results (Table 1) showed that neither the chloroquine-sensitive Honduras CDC1 strain nor the chloroquine-resistant Thailand K1 strain of parasite exhibited a stereoselective response. Taking the activity of the racemate as 1.00, the maximum ratios of the IC_{50} values for (+):(±):(−) chloroquine were 1.04:1:0.97 for the sensitive strain. For the resistant strain, the activity was approximately 10 times less than against the sensitive strain, and the stereoselectivity only marginally greater at 0.90:1:0.74.

In the case of quinacrine (Table 1) the corresponding IC_{50} ratios were 0.85:1:0.94 for the sensitive strain, again with little or no stereoselectivity. This drug showed equal activity against both strains, with the resistant strain again exhibiting little or no stereoselective behavior with IC_{50} ratios of 1.04:1:1.49. Racemic quinacrine has been previously reported to be equally active on chloroquine-resistant and chloroquine-sensitive *P. falciparum in vitro* [18].

Our results, together with the *in vitro* data reported [4] in the literature, thus confirm the absence of a significant degree of enantiomeric stereoselectivity in the *in vitro* antimalarial activity of both chloroquine and quinacrine.

It is interesting that the capacities of (+), (−) and (±) primaquine to cure infections with *P. cynomolgi* in rhesus monkeys [19] were essentially identical, and that none of the four optical isomers of the highly active piperidyl-bis (trifluoromethyl)-4-quinoline-methanol (mefloquine) (containing two asymmetric centres) showed significant differences in their activity against *P. berghei* in mice [20]. In addition, both enantiomers (the natural and its unnatural antipode) of dihydroquinine as well as those of dihydroquinidine had the same degree of activity against *P. berghei* in mice [21].

In summary, both enantiomers of quinacrine and the racemic form of the drug showed equal activity *in vitro* against chloroquine-sensitive and -resistant strains of *Plasmodium falciparum*, without detectable stereoselectivity. This contrasts with observations on chloroquine, where a similar lack of stereoselectivity *in vitro* is accompanied by a 10-fold loss of activity against the resistant strain.

The observed *in vivo* differences reported for the enantiomers of chloroquine and the observations on the optically active metabolites of chloroquine and quinacrine may therefore be ascribed to a difference in the pharmacokinetics of their enantiomers.

Table 1. Antimalarial activity of optical isomers

Strain	Isomer	Chloroquine diphosphate IC_{50} (nmol/L)	Chloroquine diphosphate 95% Confidence limits	Quinacrine dihydrochloride IC_{50} (nmol/L)	Quinacrine dihydrochloride 95% Confidence limits
CDC1	+	9.85	8.30–11.0	7.65	7.15–8.14
K1	+	127.0	102.5–157.3	3.16	2.41–4.12
CDC1	±	9.50 (9.54)*	8.14–10.66	8.98 (8.01)*	8.70–9.26
K1	±	140.6 (113.9)*	94.8–208.4	3.05 (3.73)*	2.39–3.90
CDC1	−	9.25	7.45–10.47	8.40	7.95–8.82
K1	−	103.3	90.7–117.6	4.54	3.61–5.70

Data are means with 95% confidence limits (N = 12).
* Values in parentheses are for the expected IC_{50} for racemates based on the activities for enantiomers.

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*Department of Pharmaceutical Chemistry
University of California
San Francisco
CA 94143-0446, U.S.A.
†Medical Research Institute
Pacific Presbyterian Medical Center
San Francisco
CA 94115, U.S.A.
‡Department of Medical Parasitology
London School of Hygiene & Tropical Medicine
London WC1E 7HT, U.K.

RICHARD V. WEBSTER*
JOHN C. CRAIG*
V. SHYAMALA†
GEOFFREY C. KIRBY‡
DAVID C. WARHURST‡§

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§ Corresponding author.

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