Abstract. Cross-resistance may be considered one of the most important factors leading to decreased drug susceptibility of *P. falciparum*. The study aimed to determine whether clinically relevant cross-sensitivity of *P. falciparum* existed between artemisinin and mefloquine. Seventy-six patients with falciparum malaria were admitted and treated with artemisinin derivatives. Treatment response parameters were assessed and in vitro drug sensitivity tests were performed with artemisinin, mefloquine, quinine, and chloroquine. Distinct *in vitro* cross-sensitivity between artemisinin and mefloquine was observed ($p = 0.604; P < 0.001$). To assess the relevance of this finding for clinical cross-resistance, we used an analytical model based on the relation of *in vivo* treatment response parameters (fever, parasite and symptom clearance) to a single reference drug with *in vitro* drug sensitivity data of several other drugs. Artemisinin ($R = 0.554; P = 0.009$) and mefloquine ($R = 0.615; P = 0.002$) *in vitro* drug sensitivities were equally well reflected in the *in vivo* treatment response to artemisinin, thereby suggesting the clinical relevance of *in vitro* cross-sensitivity.

**MATERIALS AND METHODS**

The study was designed to use *in vivo* and *in vitro* data acquired from patients included in controlled clinical trials at the Bangkok Hospital for Tropical Diseases (Faculty of Tropical Medicine, Mahidol University) obtained from June 1999 to March 2000. Written informed consent was obtained from all adult participants or from parents or legal guardians of minors. The study protocols were approved by the ethical review board of the Faculty of Tropical Medicine, Mahidol University.

Seventy-six patients with a median age of 24.5 years of both sexes with microscopically confirmed *P. falciparum* infections were included in the study. All patients with plasmodial infections other than *P. falciparum* as well as women who were pregnant or lactating were excluded. Patients were excluded from the study if they had a history of antimalarial drug intake before admission: artemisinin within the past 7 days, 4-aminooquinolines within the past 14 days, pyrimethamine and sulfonamides within the past 28 days, or mefloquine within the past 56 days. History of drug intake before admission was taken, and Dill and Glazko’s test for 4-aminoquinolines and the Lignin test for sulfonamides were performed to detect pretreatment.

**In vivo test procedure.** Patients were admitted to the Bangkok Hospital for Tropical Diseases and followed for 28 days to assess clinical findings, to eliminate the possibility of reinfection, and to observe cases of recrudescence. Patients were treated with a mean total dose of 11 mg/kg of artemisinin derivatives over a period of at least 3 days. Because of the large number of recrudescences to be expected with artemisinin monotherapy, artemisinin derivatives were combined with other antimalarials. To exclude a significant impact of the combination partners on the treatment response parameters, only drugs known to have a slow onset of action were used, and mefloquine was not administered before full parasite clearance. Body temperature, pulse, and respiration rates were recorded every 4 hr, and signs and symptoms were evaluated every day for the first 8 days.

Parasitological examination was performed by counting the parasites on thick and thin blood films every 12 hr. Parasite clearance time was defined as the time from the start of treatment until blood films were negative for asexual parasites of *P. falciparum* for the first time and remained negative for the next 48 hr. Fever clearance time was defined as the time from the start of treatment until the oral temperature dropped to below 37.5°C and remained below this temperature during the next 48 hr. A score of 0 to 4 (0 = none, 1 = mild, 2 = requires treatment, 3 = requires bed rest, and
Assessment of Antimalarial Cross-Resistance

Table 1
Correlation of in vitro activity at various effective concentrations for 4 antimalarial drugs*

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>EC50</th>
<th>P</th>
<th>EC90</th>
<th>P</th>
<th>EC95</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART-MEF</td>
<td>35</td>
<td>0.606</td>
<td>&lt;0.001†</td>
<td>0.580</td>
<td>&lt;0.001†</td>
<td>0.576</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>ART-QNN</td>
<td>35</td>
<td>0.206</td>
<td>&gt;0.05</td>
<td>0.084</td>
<td>&gt;0.05</td>
<td>0.063</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ART-CHL</td>
<td>35</td>
<td>0.211</td>
<td>&gt;0.05</td>
<td>0.174</td>
<td>&gt;0.05</td>
<td>0.241</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MEF-QNN</td>
<td>35</td>
<td>0.485</td>
<td>&gt;0.003†</td>
<td>0.348</td>
<td>0.004†</td>
<td>0.272</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MEF-CHL</td>
<td>35</td>
<td>0.212</td>
<td>&gt;0.05</td>
<td>0.102</td>
<td>&gt;0.05</td>
<td>0.062</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>QNN-CHL</td>
<td>35</td>
<td>0.288</td>
<td>&gt;0.05</td>
<td>0.282</td>
<td>&gt;0.05</td>
<td>0.361</td>
<td>0.03†</td>
</tr>
</tbody>
</table>

* ART = artemisinin; CHL = chloroquine; EC = effective concentration (at 50, 90 and 95%); MEF = mefloquine; p = correlation coefficient; QNN = quinine.
† Statistically significant as determined by Spearman’s rank correlation analysis.

**RESULTS**

In vitro results. The median FCT for all 76 patients was 28.0 hr. Only 3 patients showed a FCT of >100 hr, whereas 3 patients never developed fever (i.e., an oral temperature of ≥ 37.5°C). The median PCT50 was 41.0 hr (range, 19.7–65.0 hr) and the mean clearance times for 50 and 90% of the parasites (PCT50 and PCT90) were 8.6 hr (1.6–21.5 hr) and 15.3 hr (2.9–28.6 hr), respectively. The median SCT was found to be 72 hr within a range of 0–192 hr, and the median overall symptom score was 10 points (range, 0–165 points). No significant differences were found for any of these variables between the means of the 35 isolates, which were successfully tested for their in vitro drug susceptibility, as compared with the whole sample of 76 patients. The overall cure rate was 89%. Eight patients showed recrudescences within the observation period. The mean duration of time until the parasites reappeared in blood films was 17.6 ± 2.1 days (range, 15–21 days). All 8 patients showed parasite clearance within 120 hr and were therefore classified as RI cases with late recrudescence.

In vitro results. Parallel to the assessment of clinical improvement, 35 isolates were successfully tested for their in vitro susceptibility to artemisinin, mefloquine, quinine, and chloroquine. The geometric mean parasite density for these blood samples before treatment was 13,542 asexual parasites per microliter of blood. Individual ECs for all drugs were obtained by applying log-probit regression analysis to the culture results. The geometric mean of the individual EC50, EC90, and EC95 values for artemisinin were 21.0, 78.2, and 112.4 nmol/L, respectively. The corresponding values were mefloquine were 704.5, 1,718.0, and 2,211.7 nmol/L; for quinine, 357.1, 862.2, and 1,106.9 nmol/L; and for chloroquine, 2,525.0, 6,878.4, and 9,826.3 nmol/L.

In vitro correlations. The same number of isolates (n = 35) was used to correlate the individual ECs of artemisinin, mefloquine, quinine, and chloroquine at the EC50, EC90, and EC95 level, by means of Spearman’s rank correlation analysis (Table 1). Highly significant correlations with coefficients (p) as high as 0.604 (P < 0.001) were found at all EC levels between the in vitro drug susceptibility to artemisinin and mefloquine, whereas no such relation was found with quinine (ρEC90 = 0.206; P > 0.05) and chloroquine (ρEC90 = 0.211; P > 0.05). Statistically significant activity correlations were also found between quinine and mefloquine at the lower EC levels (ρEC90 = 0.485; P < 0.005) and between quinine and chloroquine at EC90 (ρEC90 = 0.361; P < 0.05).
Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC</th>
<th>n</th>
<th>df</th>
<th>R</th>
<th>Adjusted R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART</td>
<td>EC₅₀</td>
<td>35</td>
<td>31</td>
<td>0.554</td>
<td>0.240</td>
<td>0.009†</td>
</tr>
<tr>
<td>MEF</td>
<td>EC₅₀</td>
<td>35</td>
<td>31</td>
<td>0.615</td>
<td>0.319</td>
<td>0.002†</td>
</tr>
<tr>
<td>QNN</td>
<td>EC₅₀</td>
<td>35</td>
<td>31</td>
<td>0.674</td>
<td>0.402</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>CHL</td>
<td>EC₅₀</td>
<td>35</td>
<td>31</td>
<td>0.462</td>
<td>0.137</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* ART = artesinin, CHL = chloroquine, df = degrees of freedom; EC = effective concentration (at 50, 90, and 95%); MEF = mefloquine; QNN = quinine; R = correlation coefficient. ART treatment response parameters (fever, parasite, and symptom clearance times) were used as independent variables and in vitro drug sensitivity data (effective concentrations of ART, MEF, QNN, and CHL) were used as dependent variables.

† Statistically significant.

Discussion

As compared with clinical trials, in vitro drug sensitivity tests offer comparatively objective results. In vitro drug sensitivity tests are not influenced by the patient’s immune system or the formulation, absorption, or distribution of the drug, and they offer a simple way to assess cross-resistance between different antimalarials. However, relatively little is known about their clinical relevance. In this study, a combined in vivo–in vitro model was therefore used to assess the clinical relevance of in vitro cross-reactivity.

Because each of the individual treatment response parameters was found to be of limited value for the predictability of drug sensitivity, a more complex system was used, one based on a combination of FCT, PCT, and SCT. The aim was to find a quantitative measure to reflect in vivo drug sensitivity on a more comprehensive basis than individual clinical parameters. Effective concentrations were used as quantitative parameters of in vitro drug sensitivity. At the same time, the system should permit the correlation of in vivo and in vitro findings for a number of drugs other than the one tested in vivo. This rationale aimed to determine whether cross-sensitivities, which are found between in vitro results of 2 different drugs (such as between effective concentrations of artemisinin and mefloquine), are reflected in treatment response data and may therefore be of relevance for the development of clinical cross-resistance.

The naturally close correlation between artemisinin in vivo and in vitro results, which was found in the course of this analysis, reflects the dependence of drug sensitivity with therapeutic response data, a multiple regression model was used with EC values of artemisinin as dependent variable and FCT, PCT, and SCT times as the independent variables (Table 2). A significant relationship was found for all effective concentrations of artemisinin with correlation coefficients of 0.554 (P = 0.009), 0.559 (P = 0.008), and 0.578 (P = 0.005) at EC₅₀, EC₉₀, and EC₉₅, respectively. This finding reflects the close relation between results from in vitro drug sensitivity tests with artemisinin and the corresponding clinical and parasitological treatment response. The regression analysis of mefloquine in vitro culture results with the artemisinin treatment response yielded even higher correlation coefficients of 0.615 (P = 0.002), 0.674 (P < 0.001), and 0.668 (P < 0.001). These results suggest that the in vitro correlation found between artemisinin and mefloquine is also reflected in artemisinin treatment response. For quinine and chloroquine, however, no significant correlations were found, which clearly reflects the lack of a correlation between in vitro results of artemisinin on the one hand and quinine and chloroquine on the other.

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**FIGURE 1.** Model for the correlation of *in vitro* drug sensitivity of individual drugs with each other and with artemisinin treatment response parameters (arrows represent correlations, crossed-out arrows the lack of a correlation). The upper part displays the clinically relevant *in vivo* correlation between artemisinin and mefloquine. ECs = effective concentrations; FCT = fever clearance time; PCT = parasite clearance time; SCT = symptom clearance time.

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**REFERENCES**