CYTOADHERENCE CHARACTERISTICS OF \textit{PLASMODIUM FALCIPARUM} ISOLATES IN THAILAND USING AN \textit{IN VITRO} HUMAN LUNG ENDOTHELIAL CELLS MODEL

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Abstract. Using an \textit{in vitro} model of human lung endothelial cells, we studied different characteristics of \textit{Plasmodium falciparum} isolates as potential factors for malaria severity in 2 Thai patient groups: 27 with complicated malaria and 42 with uncomplicated malaria. In regard to binding properties, no association existed between cytoadherence and rosette phenotypes ($P = 0.1$) and hypothyrombocyttemia increased the cytoadherence level ($P = 0.007$). Cytoadherence was significantly associated with malaria severity ($P = 0.05$) in contrast to rosette formation ($P = 0.9$). Intercellular adhesion molecule-1 and chondroitin-4-sulfate were major receptors of cytoadherence in those with complicated malaria compared with those with uncomplicated malaria ($P < 10^{-5}$). Chondroitin-4-sulfate could act as a putative receptor for malaria complications in non-pregnant women. CD36 was the main receptor in patients with uncomplicated malaria ($P < 10^{-5}$). Vascular cell adhesion molecule-1 and E-selectin played a minor role in 2 groups ($P = 0.6$). Qinghaosu derivatives were more efficient than other antimalarial drugs, but a positive correlation was observed between the 50% inhibitory concentrations of halofantrine and quinine and the number of adhesive parasitized red blood cells, suggesting their influence on cytoadherence.

Due to the sequestration of late developmental stages of \textit{Plasmodium falciparum}-infected erythrocytes in different organs, \textit{P. falciparum} malaria can be considered as a microvascular and metabolic disease. Aside from the morphologic modifications of infected erythrocytes, there are also vasoocclusive events related to the sequestration phenomenon, especially to the cytoadherence of parasitized red blood cells (PRBCs) to the small vessel endothelium, leading to specific organ dysfunctions such as poor local perfusion, local release of cytokines and nitric oxide, and local metabolic disturbances.

The phenomenon of cytoadherence concerns the interactions between the parasite ligand \textit{P. falciparum} erythrocyte membrane protein 1 and host molecules. Different \textit{in vitro} models have proposed that at least 6 molecules that can act as adhesion receptors. These are the inducible receptors intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin and the non-inducible receptors chondroitin-4-sulfate (CSA), CD36, and thrombospondin.$^{1-8}$ In the perspective of a possible anti-sequestration therapy, knowledge of the respective roles of these putative receptors in cytoadherence could contribute to the identification of targets by which PRBCs adhere.

In a previous study, we described the efficient isolation of \textit{P. falciparum} strains with regard to 1) their profiles with respect to cytoadherence and rosette phenotypes, 2) their \textit{in vitro} chemosensitivity profiles, and 3) the interactions between these different properties and determine which factor could be considered as a marker for the severity of malaria infection.

**Patients, Materials, and Methods**

**Patients.** This study was carried out during a 2-month period from July to September 1997. The 69 patients investigated were admitted to the Hospital for Tropical Diseases in Bangkok for 28 days. All had \textit{P. falciparum} malaria: 27 cases were complicated and 42 cases were uncomplicated, according to the criteria of the World Health Organization.$^{10}$ Informed consent was obtained from all patients or their families, and the study was approved by the Ethics Committee of Mahidol University. The patients with uncomplicated malaria were treated (on day 0) with artesunate (4 mg/kg/day once a day for 3 days) in association with mefloquine (25 mg/kg in 2 doses given 6 hr apart). Those with complicated malaria were treated with artesunate (12 mg/kg over a 5-day period, 2.4 mg intravenously initially, followed by 1.2 mg/kg intravenously every 12 hr), followed by mefloquine (25 mg/kg in 2 doses given 6 hr apart when patients could tolerate oral medication). Blood samples were collected from each patient on day 0 prior to antimalarial treatment, and then once a week until day 28.

**Parasites.** Parasites from patients with a peripheral parasitemia $\geq 0.5\%$ were immediately put into culture as previously described.$^{11}$ Parasite growth was monitored by examination of Giemsa-stained thin blood films every 12 hr until each isolate reached the pigmented trophozoite stage or schizont stage. All experiments were performed during the first cycle of \textit{in vitro} parasite growth. Among the parasites from the 69 patients, only 49 isolates (23 of 27 from those with complicated malaria and 26 of 42 from those with uncomplicated malaria) grew until the late trophozoite/early schizont stages. The PRBCs were concentrated using the gelatin technique.$^{12}$ After centrifugation and a crude estimation of parasitemia, they were then adjusted at a concentration of 10% schizont stages with O group uninfected human erythrocytes in RPMI 1640 medium (Gibco, Grand Island, NY) without bicarbonate, pH 6.8, (cytoadherence medium) prior to the cytoadherence experiments.

**Target cells.** Sixth-passage HLECs were used and seeded...
at a density of 3,000 cells/well in 8-well culture plates, cultivated for 24 hr under standard conditions to reach confluence, and subsequently treated with cytokines prior to cytoadherence and cytoadherence inhibition assays. The cells were fixed for 1 hr in 200 μl of phosphate-buffered saline (PBS), 1% bovine serum albumin (BSA), 1% paraformaldehyde and kept in PBS, 0.1% BSA, 0.05% azide at 4°C for 2 weeks. For assessment of CSA, unfixed HLECs were incubated for 45 min with 0.5 IU of chondroitinase ABC/ml in RPMI 1640 medium-HEPES buffer and rinsed with PBS before being fixed with paraformaldehyde. Chinese hamster ovary cells expressing CSA without chondroitinase digestion were used as a control.

**Binding conditions of cytoadherence and cytoadherence inhibition.** To test the capacity of adhesive cells to bind *P. falciparum* isolates (cytoadherence phenotype) and to define the specificity of each receptor, all cytoadherence and cytoadherence competition tests were performed as previously described in the presence or absence of 20 μg/ml of monoclonal antibodies against CD36 (OKM5; Ortho Diagnostic Systems, Raritan, NJ) ICAM-1, VCAM-1, and E-selectin (R & D Systems Europe, Ltd., Abingdon, United Kingdom), and CSA (Boehringer Ingelheim Bioproducts, Gagny, France) used alone or in combination. All cytoadherence and cytoadherence inhibition tests were performed in triplicate. A suspension of PRBCs at a 10% parasitemia and a 5% hematocrit was washed in RPMI 1640 medium for 1 hr at room temperature with continuous, gentle shaking. After removal of non-adherent erythrocytes with 3 washes using cytoadherence medium, the preparation was fixed for 60 min with 2% glutaraldehyde and rinsed with PBS.

The binding of Giemsa-stained PRBCs to 200 cells was counted in a light microscope by 2 observers in a blind fashion. Cytoadherence was expressed as the number of PRBCs cultivated with and without monoclonal antibodies, expressed as a percentage value with its confidence interval.

**Rosette formation.** The rosette assay was performed using 100 μl of a fresh PRBC suspension of each isolate mixed with 2 μl of 0.01% acridine orange solution. Ten microliters of the suspension were placed under a coverslip. One hundred PRBCs were counted with a fluorescence microscope. A rosette was scored if 2 or more uninfected cells were bound to a single, infected erythrocyte. The extent of rosette formation was calculated as the ratio between cytoadherence of PRBCs cultivated with and without monoclonal antibodies, expressed as a percentage value with its confidence interval.

**Chemosensitivity profile and effects of antimalarial drugs on cytoadherence.** To determine the chemosensitivity profiles, in vitro sensitivity tests against 8 antimalarial drugs, namely chloroquine (CQ), quinine (QN), mefloquine (MF), halofantrine (HF), artemisinin (A1), artemether (AM), artesunate (AU), and atovaquone (AQ), were carried out as previously described. Among the 69 patients initially included, viable cultures were obtained from only 49 patients (23 with complicated malaria and 26 with uncomplicated malaria). In addition, the results of the isotopic chemosensitivity tests were exploitable for 28 patients (14 with complicated malaria and 14 with uncomplicated malaria). The results of the different 50% inhibitory concentrations (IC50s) were compared for 5 antimalarial drugs with known thresholds (CQ, QN, MF, HE, and AQ) as previously described. However, to study the interference of 3 antimalarial drugs on cytoadherence, PRBCs were kept in culture for 24–36 hr in the presence of 1 mg/ml of QN, 26 ng/ml of HF, and 49 ng/ml of AM. The control parasite cultures (parasite cultures without antimalarial drugs) were incubated for the same duration as those with antimalarial drugs. These concentrations were determined in our previous study as having a subinhibitory effect on the parasite growth. Cytoadherence assays and the binding of Giemsa-stained PRBCs to 200 cells were performed as above. The percentage value of cytoadherence inhibition were calculated for each antimalarial drug as the ratio between cytoadherence of PRBCs cultivated with antimalarial drugs and those cultivated without antimalarial drugs.

**Statistical analysis.** Because the data were not normally distributed, we compared the variance of the distribution of different items using the Kruskal-Wallis test in the 2 groups. The association between cytoadherence and rosette profiles was analyzed using the chi-square test. Spearman correlation analysis was done to determine the interactions between the quantitative items. Differences were considered significant when the *P* value was ≤0.05.

**RESULTS**

**Clinical and biological characteristics.** The clinical and biological characteristics of patients are summarized in Table 1. Both groups were similar in terms of age (range = 13–50 years for those with complicated malaria and 14–50 years for those with uncomplicated malaria) and sex. No mortality was observed during the period of the study.

**Cytoadherence phenotype.** Forty-nine isolates (from 23 of 27 cases with complicated malaria and 26 of 42 with uncomplicated malaria) grew until late trophozoites/early schizont stages and were analyzed. However, there was no significant difference in parasite cultures from the 2 different disease categories (*P* = 0.4). Among them, 17 (74%) of 23 from cases with complicated malaria had a cytoadherence phenotype compared with 14 (54%) of 26 cases with uncomplicated malaria. This difference was statistically significant, indicating an association between cytoadherence phenotype and malaria severity (*P* = 0.05). When compared with cases with uncomplicated malaria, a cytoadherence phenotype was 3.31 times more common in those with complicated malaria (odds ratio = 3.3, 95% confidence interval = 0.9–13.4, *P* = 0.03). However, the mean ± SD number of PRBCs adhered to 200 HLECs in the cases with complicated malaria (106 ± 184) was significantly greater than that in those with uncomplicated malaria (12 ± 22, *P* = 0.05).

**Cytoadherence competition tests.** Cytoadherence was selectively reduced with a varying efficiency in the presence of monoclonal antibodies. In case with complicated malaria,
binding of PRBCs via ICAM-1, CSA, VCAM-1, E-selectin, and CD36 was observed in 94%, 93%, 49%, 34%, and 25%, respectively, of all isolates (Table 2). Intercellular adhesion molecule-1 and CSA were the major receptors by which isolates from complicated malaria cases adhered to HLECs compared with those with uncomplicated malaria (ICAM-1 = 37%, CSA = 39%; P < 10⁻⁴), whereas VCAM-1 and E-selectin participated to lesser degree. In cases with uncomplicated malaria, the binding of PRBCs via CD36 (76%) was higher than in those with complicated malaria (P < 10⁻³), indicating that this receptor is not associated with malaria severity.

Effects of antimalarial drugs on cytoadherence. Quinine, AM, and HF inhibited adherence of PRBCs to HLECs. The inhibitory effect of AM was more potent than the effects of QN and HF. No significant difference was observed between the 2 groups (Table 3).

Rosette formation. Thirty-seven isolates were studied for the rosette phenotype in the 2 groups (16 cases with complicated malaria and 21 with uncomplicated malaria) for the reasons mentioned earlier. Sixty-nine percent of those with complicated malaria had the rosette phenotype compared to 71% of those with uncomplicated malaria (P < 10⁻³), indicating that this receptor is not associated with malaria severity.

Chemosensitivity profile. The chemosensitivity tests results for the 49 isolates with viable cultures were interpretable for only 28 cases (14 with complicated malaria and 14 with uncomplicated malaria) for the reasons mentioned earlier. There was evidence of chloroquine resistance in all isolates and resistance to HF in cases with complicated malaria, whereas all isolates were sensitive to ME, AQ, and to 3 qinhaosu derivatives (AI, AM, and AU). We observed a low sensitivity of all P. falciparum isolates to quinine compared with the cut-off value. A significant difference was observed between the IC₅₀ of the isolates from the 2 groups for HF (P = 0.04), AI (P = 0.02), and AM (P = 0.02) (Table 4). Correlations between different antimalarial drugs are shown in Table 5. No significant correlation was observed between AQ and the other 7 drugs. There was a significant and positive correlation between CQ and QN, QN and AI, QN and HF, CQ and AI, HF and AI, and between the different qinhaosu derivatives (AM and AU and AI and AU).

Cytoadherence, rosette, and chemosensitivity phenotypes of P. falciparum isolates. Our data showed a strong positive correlation between the rosette formation and the IC₅₀ of AI, CQ, and HF (Table 6). There was a significant correlation between adhesive PRBCs and the in vitro chemosensitivity profile of the isolates for QN and HF. We did not observe the same results for the other antimalarial drugs (Table 7). A significant negative correlation was observed between the cytoadherence phenotype and thrombocytopenia (r = −0.42, P = 0.007, n = 49), but no association was observed between hemoglobin levels, cytoadherence, and rosette formation. Analysis of rosette formation and cytoadherence profiles showed no significant association (P = 0.1, n = 37) (Table 8).

DISCUSSION

The present study investigated several characteristics of P. falciparum in terms of quantitative bindings studies using a new in vitro cytoadherence model, HLECs, and fresh Thai isolates. We also studied the chemosensitivity of isolates to

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**TABLE 1**
Clinical and biologic characteristics of the patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Complicated malaria</th>
<th>Uncomplicated malaria</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>23</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>15</td>
<td>0.1</td>
</tr>
<tr>
<td>Mean (SD) Age (years)</td>
<td>26 (10)</td>
<td>25 (8)</td>
<td>0.9</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10 (3.3)</td>
<td>11 (2.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>Red blood cells (10⁶ × 10¹² L)</td>
<td>3.8 (1.3)</td>
<td>4 (1)</td>
<td>0.7</td>
</tr>
<tr>
<td>Platelets count (platelets/µl)</td>
<td>79,227 (59,733)</td>
<td>93,320 (58,095)</td>
<td>0.4</td>
</tr>
<tr>
<td>Blood glucose (g/L)</td>
<td>1.3 (0.2)</td>
<td>1.3 (0.2)</td>
<td>0.5</td>
</tr>
<tr>
<td>Parasitemia (parasites/µl)</td>
<td>241,440 (315,540)</td>
<td>66,195 (103,261)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* By the Kruskal-Wallis test in both complicated and uncomplicated malaria cases.

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**TABLE 2**
Percent inhibition of parasitized red blood cells (PRBCs) having adhered to 200 human lung endothelial cells (HLECs) in the presence of various monoclonal antibodies

<table>
<thead>
<tr>
<th>Isolate profile*</th>
<th>Complicated cases (n = 20)</th>
<th>Uncomplicated cases (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Inhibition</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Cytoadherence</td>
<td>106.2 (84)</td>
<td>94%</td>
<td>11.6 (21.6)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>6.6 (8.4)</td>
<td>94%</td>
<td>7.3 (15.8)</td>
</tr>
<tr>
<td>CSA</td>
<td>7.9 (9.3)</td>
<td>93%</td>
<td>7.1 (15.8)</td>
</tr>
<tr>
<td>E-selectin</td>
<td>69.8 (124)</td>
<td>34%</td>
<td>5.9 (10.9)</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>54.7 (99.6)</td>
<td>49%</td>
<td>6.3 (11.8)</td>
</tr>
<tr>
<td>CD36</td>
<td>80.2 (146.2)</td>
<td>25%</td>
<td>2.8 (5.6)</td>
</tr>
</tbody>
</table>

* ICAM-1 = intercellular adhesion molecule-1; CSA = chondroitin-4-sulfate; VCAM-1 = vascular cell adhesion molecule-1.
† Calculated based on the number of PRBCs having adhered to 200 HLECs by using the Kruskal-Wallis test in both complicated and uncomplicated malaria cases.
‡ Calculated based on the difference in % inhibition by using the chi-square test in both complicated and uncomplicated malaria cases.
8 antimalarial drugs to determine the correlation between cytoadherence properties and antimalarial drug sensitivity and to examine the effect of antimalarial drugs on the cytoadherence properties. Our results may provide useful information about the interactions between these different factors.

Previous studies have shown that there is a wide range of binding affinities in different parasites. We observed an association between cytoadherence phenotype and malaria severity. In addition, there was a higher affinity of Thai isolates for ICAM-1 and CSA from cases with complicated malaria than in those from cases with uncomplicated malaria. It is not possible to ascertain whether a patient with uncomplicated malaria will develop severe complications.

We examined quantitatively the ability of 36 isolates to adhere to ICAM-1, CSA, VCAM-1, E-selectin, and CD36. The results indicated that in those with complicated malaria, approximately 93% of the isolates showed significant adherence to ICAM-1 and CSA. This binding was higher than that observed in the cases with uncomplicated malaria (approximately 38% of the isolates). Previous studies by other investigators reported the role of ICAM-1 in in vitro cytoadherence using cell models originating from target organs of P. falciparum and in cases of fatal malaria. Robert and others found CSA on the surface of HLECs. This result is surprising since another study suggested that this molecule was responsible for sequestration of parasites in pregnant women with malaria. Our data suggest that CSA could act as a receptor in non-pregnant malaria cases. However, our findings on the importance of CSA and ICAM-1 in the course of malaria infection contrasts with those of other investigators who reported that the level of adherence of P. falciparum isolates to C32 melanoma cells was similar for ICAM-1 and CSA, but lower than for CD36.

In cases with uncomplicated malaria, binding to CD36 was higher (76%) than those with complicated malaria (25%). Our data are consistent with a role for enhanced binding to ICAM-1 and CSA in the development of complicated malaria, and are not consistent with a role for enhanced binding to CD36. The high-affinity binding to CD36 in cases with uncomplicated malaria suggests that abolishing CD36-dependent adhesion could lead to the selection of parasites with affinities for receptors such as ICAM-1 or CSA, which may result in increased cytoadherence. With regards to the CSA receptor, further research is needed to determine whether in pregnant women with malaria P. falciparum induces a similar, specific response to a wide range of CSA-binding parasites. More information is required to establish which of the host receptors are critical in the development of severe malaria.

Binding of isolates to VCAM-1 and E-selectin was low, and there was no significant difference between the 2 groups. As reported by others, VCAM-1 and E-selectin may play a minor role in sequestration.

In general, adherence properties may depend on the methodologic techniques used, the in vitro models used, and the host immunologic status. With the identification of so many receptors and such a wide spectrum of clinical outcomes in relation with host properties, studies are needed to establish an association between the ability of parasites to bind to a given receptor and the disease.

In the present study, no association was observed between rosette formation and malaria severity. The role of rosette formation in the pathogenesis of severe malaria is complex and still controversial. Most but not all studies that have examined the relationship between rosette formation and disease suggest that parasites with a rosette formation pheno-

<table>
<thead>
<tr>
<th>Table 3</th>
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<tbody>
<tr>
<td><strong>In vitro drug sensitivity test results for the different Plasmodium falciparum isolates</strong></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs†</th>
<th>Uncomplicated cases (n = 14)</th>
<th>Complicated cases (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage of resistance</td>
<td>Median IC\textsubscript{50} (nmol/L)</td>
</tr>
<tr>
<td>CQ</td>
<td>50%</td>
<td>115</td>
</tr>
<tr>
<td>QN</td>
<td>17%</td>
<td>94</td>
</tr>
<tr>
<td>MQ</td>
<td>0%</td>
<td>3.8</td>
</tr>
<tr>
<td>HF</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>AI</td>
<td>ND</td>
<td>1.7</td>
</tr>
<tr>
<td>AM</td>
<td>ND</td>
<td>1.9</td>
</tr>
<tr>
<td>AU</td>
<td>ND</td>
<td>0.4</td>
</tr>
<tr>
<td>AQ</td>
<td>0%</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* IC\textsubscript{50} = 50% inhibitory concentration.
† CQ = chloroquine; QN = quinine; MQ = mefloquine; HF = halofantrine; AI = artesimin; AM = artemether; AU a = artesunate; AQ = atovaquone.
‡ Calculated by using the Kruskal-Wallis test in both complicated and uncomplicated malaria cases.

In cases with uncomplicated malaria, binding to CD36 was higher (76%) than those with complicated malaria (25%). Our data are consistent with a role for enhanced binding to ICAM-1 and CSA in the development of complicated malaria, and are not consistent with a role for enhanced binding to CD36. The high-affinity binding to CD36 in cases with uncomplicated malaria suggests that abolishing CD36-dependent adhesion could lead to the selection of parasites with affinities for receptors such as ICAM-1 or CSA, which may result in increased cytoadherence. With regards to the CSA receptor, further research is needed to determine whether in pregnant women with malaria P. falciparum induces a similar, specific response to a wide range of CSA-binding parasites. More information is required to establish which of the host receptors are critical in the development of severe malaria.

Binding of isolates to VCAM-1 and E-selectin was low, and there was no significant difference between the 2 groups. As reported by others, VCAM-1 and E-selectin may play a minor role in sequestration.

In general, adherence properties may depend on the methodologic techniques used, the in vitro models used, and the host immunologic status. With the identification of so many receptors and such a wide spectrum of clinical outcomes in relation with host properties, studies are needed to establish an association between the ability of parasites to bind to a given receptor and the disease.

In the present study, no association was observed between rosette formation and malaria severity. The role of rosette formation in the pathogenesis of severe malaria is complex and still controversial. Most but not all studies that have examined the relationship between rosette formation and disease suggest that parasites with a rosette formation pheno-
type are more likely to occur in patients with severe disease.\textsuperscript{26,27} This difference might be due to the methodologic variations and intrinsic differences attributable to the geographic origin of the parasites.\textsuperscript{14,28} However, it is well established that the frequency of rosette formation is diminished when parasites are growing in cells exhibiting hemoglobinopathies known to be protective against severe malaria.\textsuperscript{29} Despite the absence of an association between this phenotype and the severity of infection in this study, rosettes could reflect an additional binding phenotype that may be associated with a poor outcome of malaria infection.

*Plasmodium falciparum* resistance to antimalarial drugs in non-immune subjects may constitute a virulence factor when compared with forms of malaria due to chemosensitive strains. In this study, we observed a significant difference between the IC\textsubscript{50} of the isolates from the 2 of patients for HF, AI, and AM, suggesting that the chemosensitivity profile can be used as a marker of severity of *P. falciparum* malaria. Our results support findings that all isolates from Thailand showed chloroquine resistance and that QN is not an ideal antimalarial agent.\textsuperscript{30} Thai isolates responsible for complicated malaria were resistant to HF while those responsible for uncomplicated malaria were sensitive to this drug. This result confirms a well-known inadequacy of HF when in monotherapy in the southeast Asian region.\textsuperscript{31} We observed higher IC\textsubscript{50}s for qinghaosu derivatives for isolates from cases with complicated malaria compared with those from cases with uncomplicated malaria, with a significant correlation between QN and HF. Our results confirm the results of monotherapy with uncomplicated malaria were sensitive to this drug. This result confirms the results of monotherapy with *P. falciparum* malaria to avoid the appearance of drug resistance. The use of AQ and proguanil in combination is advised because of the high sensitivity of all isolates to AQ. There was no significant correlation between this drug and other 7 antimalarial drugs tested. In the experiments testing cytoadherence inhibition by antimalarial drugs, no significant differences were observed between the 2 malaria groups. Nevertheless, AM reduced cytoadherence to a greater extent than QN and HF. Our results confirm the results of clinical trials that reported the efficiency of AM compared with QN for treatment of cerebral malaria.\textsuperscript{32,33} Previous studies have also shown inhibition of cytoadherence by antimalarial drugs, with the greatest inhibitory activity cytoadherence correlated with the efficacy against severe malaria.\textsuperscript{34}

One of the important aspects of this work was the simultaneous study of potential pathogenic factors to determine their interactions. In the course of this study, a strong correlation was found between the IC\textsubscript{50} of HF and the number of PRBCs adhering to HLECs, and between the IC\textsubscript{50} of HF and the number of PRBCs that formed rosettes. These associations suggest that strains resistant to HF have a tendency to cytoadhere and form rosettes. This may be an essential,

\begin{table}[h]
\centering
\caption{Correlation between antimalarial drugs among \textit{Plasmodium falciparum} isolates from uncomplicated and complicated cases*}
\begin{tabular}{|c|c|c|c|}
\hline
Drugs & n & r & P  \\
\hline
AM \times AU & 28 & 0.76 & 0.001  \\
QN \times HF & 28 & 0.70 & 0.003  \\
HF \times AI & 26 & 0.76 & 0.004  \\
CQ \times AI & 24 & 0.74 & 0.005  \\
AI \times AU & 26 & 0.62 & 0.02  \\
CQ \times QN & 28 & 0.52 & 0.04  \\
QN \times AI & 24 & 0.59 & 0.04  \\
CQ \times HF & 28 & 0.49 & 0.06  \\
HF \times MQ & 28 & 0.49 & 0.06  \\
AI \times AM & 26 & 0.46 & 0.1  \\
AQ \times AU & 28 & 0.4 & 0.17  \\
QN \times MQ & 28 & 0.36 & 0.18  \\
HF \times AM & 26 & 0.37 & 0.21  \\
MQ \times AI & 26 & 0.36 & 0.21  \\
QN \times AM & 26 & 0.32 & 0.27  \\
MQ \times AM & 26 & 0.31 & 0.28  \\
CQ \times MQ & 26 & -0.02 & 0.90  \\
CQ \times MQ & 28 & 0.01 & 0.94  \\
\hline
\end{tabular}
\textsuperscript{*} Spearman correlation analysis.
\textsuperscript{†} HF = halofantrine; QN = quinine; HF = halofantrine; AI = artemisinin; AM = artemether; AU = artemate; AQ = atovaquone.
\end{table}

\begin{table}[h]
\centering
\caption{Correlation between adhesive parasitized red blood cells to 200 human lung endothelial cells and \textit{in vitro} drug sensitivity test results*}
\begin{tabular}{|c|c|c|c|}
\hline
Isolate profile & n & r & P  \\
\hline
Cytoadherence \times HF & 26 & 0.70 & 0.007  \\
Cytoadherence \times QN & 26 & 0.60 & 0.03  \\
Cytoadherence \times CQ & 26 & 0.48 & 0.08  \\
Cytoadherence \times AI & 22 & 0.44 & 0.16  \\
Cytoadherence \times MQ & 26 & 0.24 & 0.42  \\
Cytoadherence \times AU & 22 & 0.13 & 0.70  \\
Cytoadherence \times AM & 22 & -0.09 & 0.77  \\
Cytoadherence \times AQ & 22 & 0.09 & 0.78  \\
\hline
\end{tabular}
\textsuperscript{*} Spearman correlation analysis.
\textsuperscript{†} HF = halofantrine; QN = quinine; CQ = chloroquine; AI = artemisinin; MQ = mefloquine; AU = artesunate; AM = artemether; AQ = atovaquone.
\end{table}

\begin{table}[h]
\centering
\caption{Correlation between the number of rosettes and \textit{in vitro} drug sensitivity test results*}
\begin{tabular}{|c|c|c|c|}
\hline
Isolate profile & n & r & P  \\
\hline
Rosette \times AI & 24 & 0.74 & 0.005  \\
Rosette \times CQ & 26 & 0.70 & 0.007  \\
Rosette \times HF & 26 & 0.69 & 0.008  \\
Rosette \times AU & 24 & 0.53 & 0.07  \\
Rosette \times QN & 26 & 0.49 & 0.08  \\
Rosette \times MQ & 26 & 0.29 & 0.33  \\
Rosette \times AQ & 24 & 0.16 & 0.60  \\
Rosette \times AM & 24 & 0.09 & 0.76  \\
\hline
\end{tabular}
\textsuperscript{*} Spearman correlation analysis.
\textsuperscript{†} AI = artesminin; CQ = chloroquine; HF = halofantrine; AU = artesunate; QN = quinine; MQ = mefloquine; AQ = atovaquone; AM = artemether.
\end{table}

\begin{table}[h]
\centering
\caption{Correlation between the different parameters: numbers of hemoglobin, platelet, adhesive parasitized red blood cells to 200 human lung endothelial cells and rosettes*}
\begin{tabular}{|c|c|c|c|}
\hline
Isolate profile & n & r & P  \\
\hline
Cytoadherence \times platelets & 49 & -0.42 & 0.007  \\
Rosette \times cytoadherence & 37 & 0.32 & 0.08  \\
Cytoadherence \times hemoglobin & 49 & 0.28 & 0.08  \\
Rosette \times platelets & 37 & -0.14 & 0.41  \\
Rosette \times hemoglobin & 37 & 0.08 & 0.62  \\
\hline
\end{tabular}
\textsuperscript{*} Spearman correlation analysis.
\textsuperscript{†} HF = halofantrine; QN = quinine; CQ = chloroquine; MQ = mefloquine; AU = artesunate; AM = artemether; AQ = atovaquone.
\end{table}
though not necessarily sufficient, condition for the eventual outcome of the disease. On the other hand, these results show no association between cytoadherence and rosette formation, suggesting once again that rosette formation is not a characteristic of strains that cause severe malaria. This conclusion is further justified by the fact that rosette formation is found in the course of malaria caused by other plasmodial species. Among the interactions between the different characteristics and the biological and clinical parameters, a strong negative correlation was found between the number of PRBCs and the number of platelets. Grau and others have suggested a protective effect against murine cerebral malaria by thrombocytopoiesis induced by injection of a monoclonal antibody to leukocyte function antigen-1.

With this new in vitro system (the HLECs), it has been shown that cytoadherence is a characteristic of isolates responsible for complicated malaria in Thailand. The respective roles of the principal receptors implicated in this phenomenon (ICAM-1, CSA, E-selectin, VCAM-1, and CD36) has also been described. No association has been observed between the cytoadherence profile and rosette formation, confirming the absence of association between the latter and the severity of malaria. Conversely, these results have shown a strong correlation between the IC₅₀ of AI, HF, and CQ and the number of PRBCs forming rosettes, and a strong correlation between the number of PRBCs having adhered to the HLECs and the IC₅₀ of HF and of QN. A very strong negative correlation between the number of circulating platelets and the number of PRBCs adhering to HLECs has also been shown. These correlations shed light on the role of platelets in the physiopathology of severe malaria, and underline the possibility of treating the disease with either anti-receptor antibodies or with antibodies that block the sequestration of platelets. Other mechanisms, such as the immune response of the host, the role of soluble mediators (nitric oxide), and the role of cytokines implicated in the physio-pathology of malaria, have to be taken into account or studied with the different characteristics just described to develop a strategy for effectively treating complicated malaria or preventing its onset.

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