PENTOXIFYLLINE AS AN ANCILLARY TREATMENT FOR SEVERE FALCIPARUM MALARIA IN THAILAND


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Abstract. Pentoxifylline, an inhibitor of tumor necrosis factor, has been evaluated as an antimalarial agent in combination with artesunate in 45 patients with severe falciparum malaria. Patients were admitted to the intensive care unit at the Hospital for Tropical Diseases in Bangkok, Thailand, and randomly assigned to treatment for 72 hr with a combination of intravenously administered artesunate and 1) placebo, 2) low-dose pentoxifylline (0.83 mg/kg/hr), or 3) high-dose pentoxifylline (1.67 mg/kg/hr). All 45 patients had one or more manifestations of severe malaria such as cerebral malaria (n = 18), renal failure requiring hemodialysis (n = 9), azotemia (n = 8), jaundice (n = 25), or hyperparasitemia (n = 30). The overall severity was comparable in the three groups. Clinical outcome was assessed with respect to the parasite clearance time and the fever clearance time in all patients. In addition, a number of subsidiary outcome variables were examined in specific subgroups, including the recovery time from coma for patients with cerebral malaria, the duration of intubation in patients with respiratory distress, the number of hemodialysis treatments needed for patients with acute renal failure, and the number of units of blood administered to patients requiring transfusion. Concentrations of tumor necrosis factor were reduced in all three groups at 48 hr after treatment. No significant differences among the three treatment groups were found for any of the outcome variables examined. We conclude that the addition of pentoxifylline to artesunate therapy for severe malaria produced no evident clinical benefit.

The essential pathologic feature of severe malaria is the sequestration of erythrocytes containing advanced and mature asexual parasite forms in deep vascular beds in internal organs. Sequestration is greatest in the brain, which probably explains why cerebral malaria is the most common manifestation of severe malaria. Sequestration results in the obstruction of the microcirculation leading to hypoxia, accumulation of lactic acid, and generation of free oxygen radicals. Three distinct processes may interfere with microcirculatory flow; namely, cytoadherence to vascular endothelium, binding of uninfected erythrocytes to erythrocytes infected with mature parasites (rosette formation), and decreased deformability of infected red blood cells.

Elevated levels of circulating tumor necrosis factor-α (TNF-α) are correlated with disease severity in falciparum malaria and sepsis.1-3 High TNF-α levels also correlate with mortality in cerebral malaria in African children1 and in severe septicemia.4 It has been postulated that TNF-α might exacerbate the sequestration of infected red blood cells to cerebral vessels by up-regulating adhesion molecules, such as intercellular adhesion molecule-1, which mediate the binding of the infected parasitized erythrocytes to vascular endothelium.5 In experimental malaria in animals, excessive production of TNF was found to promote cerebral malaria.6 Although these observations and postulates are of interest, a pathogenetic role for TNF-α in human cerebral malaria has not been established.

Pentoxifylline may ameliorate the course of falciparum malaria by inhibiting the expression of adhesion molecules through down-regulation of the proinflammatory cytokines TNF-α and interleukin-1 (IL-1), thereby decreasing attachment of the infected red blood cells to the vessel wall. This compound may also inhibit red blood cell aggregation and increase the deformability of red blood cells by influencing intracellular adenine nucleotide levels, calcium ions, and inhibiting the cAMP-independent protein kinase.

Pentoxifylline inhibits synthesis via the inhibition of phosphodiesterase and the increase of intracellular cAMP. In a laboratory model, pentoxifylline depressed TNF-α production by macrophages at the transcriptional level in a dose-dependent manner and reduced TNF-α supernatant bioactivity.7 By reducing levels of TNF-α, pentoxifylline could have a beneficial effect on complications thought to involve this cytokine,7-11 e.g., acute respiratory distress syndrome,12,13 renal failure,14,15 and cerebral malaria.16,17 Pentoxifylline has also been shown to increase the deformability of red blood cells, thereby in part potentially of offsetting the decreased deformability of infected erythrocytes.18,19 It also has an effect on rosette formation in vitro by inhibiting red blood cell aggregation.20 Studies in mice and in human malaria have suggested that pentoxifylline may have a beneficial effect in the treatment of severe malaria.16,17,21 We report here a clinical study on the use of pentoxifylline as an ancillary treatment in an open, randomized study in 45 patients with severe falciparum malaria.

PATIENTS AND METHODS

Patients admitted to the Bangkok Hospital for Tropical Diseases between January and May 1994, were accepted into the study if they were diagnosed as having severe falciparum malaria as defined by the world Health Organization,22 were more than 16 years old, weighed more than 45 kg, and if informed consent to take part in the study was provided by the patient or relatives. Reasons for exclusion were pregnancy, underlying diseases that had been treated with pentoxifylline, or a history of allergy to pentoxifylline. This study
was approved by the Ethics Committee of Mahidol University.

All patients were admitted to the intensive care unit of the hospital for at least 72 hr for careful observation and were then transferred to general wards after their condition had improved. Patients then remained in the hospital for 28 days to assess clinical outcome, safety, and tolerance and to evaluate the cure rate at 28-day follow-up. Body temperature, pulse, and respiratory rates were measured every 4 hr during the first seven days and then twice a day for the remainder of the study. Blood pressure was measured every 4 hr during the pentoxifylline infusion, then once a day until the seventh day and weekly thereafter. Clinical signs and symptoms were evaluated every day for the first seven days and weekly thereafter. Side effects were defined as signs and symptoms that occurred or became more severe after treatment had started. Cure was defined as the absence of recrudescent parasitemia during the 28-day follow-up.

Pretreatment investigations included full blood count, electrolytes, total and direct bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, albumin, globulin, aspartate and alanine aminotransferases, and urine analysis. These tests were repeated daily as indicated clinically during the comatose period and then on days 3, 7, 14, 21, and 28. Thick blood films were examined for parasites every 6 hr during treatment until they were found to be negative, and then once a day. The films were considered negative if no parasites were seen in 200 oil-immersion microscopic fields. Parasite clearance time was taken as the period from the start of treatment until the first negative blood film. Fever clearance time was taken as the period from the start of treatment until the oral temperature decreased to 37.5°C and remained below this temperature for the next 48 hr. The percentage of parasitemia after the onset if treatment was the proportion of current to initial parasitemia × 100.

**Treatment.** All patients were treated with artesunate (120 mg intravenously initially followed by 60 mg intravenously every 12 hr; total dose = 600 mg) and were openly randomized into three groups on the basis of precoded sealed envelopes. Group III was given a high dose of pentoxifylline (1.67 mg/kg/hr). Group II was given a low dose of pentoxifylline (0.83 mg/kg/hr). Group I was given placebo (0.9% NaCl, 1 ml/kg/hr). Pentoxifylline or placebo were administered in a continuous, rate-controlled infusion for the first 72 hr of treatment. The high-dose regimen has been used previously as an ancillary treatment for cerebral malaria. Clinical evaluation was based on mortality, the clinical resolution of complications such as recovery from coma, renal failure, and respiration failure and fever and parasite clearance times.

Blood samples for cytokine assay were obtained before and after initiation of treatment at 6 and 12 hr and on days 1, 2, 7, 14, 21, and 28. Details of these findings are reported in a companion paper.

**Statistical analysis.** Normally distributed data for the three groups were compared by analysis of variance (ANOVA). Proportional data were tested by the chi-square test.

### Results

Forty-five patients were enrolled in the study. Baseline characteristics of patients and pretreatment laboratory data are shown in Table 1. The majority of the patients (78%) had malaria for the first time and had contracted the infection on the Thailand-Myanmar border. The severity of patient complications is shown in Table 2. Eighteen patients (40%)
had cerebral malaria with a mean Glasgow coma score of 8 (range = 5–11). Nine patients had acute renal failure with anuria requiring hemodialysis, while eight patients had serum creatinine and/or blood urea nitrogen levels more than three times above normal but these returned to normal after treatment. Twenty-five patients were jaundiced and 24 patients had serum transaminase levels more than three times above normal. Thirty patients had hyperparasitemia (more than 2% of the erythrocytes infected). The three groups were similar before treatment (P > 0.05, by ANOVA).

**Response to treatment.** After treatment, all patients showed major clinical improvement within 3–7 days and no patient died. Patients in groups II and III tolerated pentoxifylline well and were similar to Group I. There were no manifested adverse effects. Minor complaints were reported by one or two patients in each group, consisting of headache, dizziness, and nausea. They were equally distributed in the three groups. Most of these complaints were recorded on the first three days of treatment and generally coincided with high fever, but it was not possible to determine whether these were due to malaria or related to the drugs. During the 28 days of hospitalization, there were no neurologic or neuropsychiatric manifestations. The results of serial laboratory tests were unremarkable, and they usually returned to normal levels within 2–3 weeks. There were no evident differences among the groups. One patient in Group I had hemoglobinuria on admission but recovered on day 3 after treatment. This patient had glucose-6-phosphate dehydrogenase–deficient red blood cells.

Two patients, one each in Groups II and III, were fully conscious (Glasgow Coma Score = 15) on admission, but showed altered consciousness (Glasgow Coma Scores = 9 and 10) after 6–12 hr of initial treatment. Both regained consciousness in 1–2 days after continuing treatment. Patients with acute renal failure required hemodialysis 4–5 times during 5–7 days to restore kidney function; no differences among the groups were found with respect to the duration of therapy or the number of treatments required. A high proportion of patients in all three groups were anemic and the mean units of blood given per patient were 3.3, 5.1, and 3.7 in Groups I, II, and III, respectively. A similar number of patients in the groups developed adult respiratory distress syndrome and required ventilation and were extubated within 1–3 days (Table 2). Elevated levels of liver enzymes returned to normal levels in (median) seven, eight, and eight days after the start of treatment in Groups I, II, and III, respectively.

The mean parasite clearance times were 59.2, 60.8, and 57.6 hr in Groups I, II, and III, respectively, and there were no significant differences among the groups. The times to 50% and 90% reduction also showed no significant differences among the groups (Figure 1). The mean fever clearance time was longer in the group given a high dose of pentoxifylline but this did not reach a statistically significant level (P > 0.05). If patients with concomitant infections (sepsis) were excluded from the calculation (n = 36), the mean fever clearance times were similar.

Fourteen patients dropped out of the study after treatment (median = 13 days, range = 5–23 days) for social reasons and not due to drug-related adverse effects (Table 3). These patients had cleared symptoms, fever, and parasitemia and reached normal laboratory parameters at the time of discharge from the hospital. All groups showed a similar cure rate at 28 days (50%, 67%, and 56% in Groups I, II, and III, respectively) after treatment with 600 mg of artesunate over a five-day period. Twelve patients who had RI recurrence between 16 and 23 days after treatment were successfully treated with quinine plus tetracycline for seven days.

**DISCUSSION**

Severe falciparum malaria carries a high mortality rate that usually varies between 10% and 40%, depending upon

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**Table 2**

Severity and clinical resolution of patient complications

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 15)</th>
<th>Group II (n = 15)</th>
<th>Group III (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral malaria</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Glasgow coma score, range</td>
<td>6–11</td>
<td>6–10</td>
<td>5–11</td>
</tr>
<tr>
<td>Median (days) to regain consciousness</td>
<td>2.8</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Range</td>
<td>1–6</td>
<td>3–6</td>
<td>1–6</td>
</tr>
<tr>
<td>Acute renal failure (required hemodialysis)</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Median of hemodialysis/patient†</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Median duration (days) of hemodialysis/patient†</td>
<td>5</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Azotemia‡</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Jaundice</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Increased serum transaminase enzyme level§</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Anemia</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>No. of patients needing blood transfusion</td>
<td>23</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>Mean units of blood transfusion/patient</td>
<td>3.3</td>
<td>5.1</td>
<td>3.7</td>
</tr>
<tr>
<td>No. of patients requiring intubation</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Duration of intubation (days)</td>
<td>1–2</td>
<td>1–2</td>
<td>1–3</td>
</tr>
</tbody>
</table>

* For definitions of groups, see Table 1.
† Not significant between the groups.
‡ Increased creatinine and/or blood urea nitrogen more than three times normal levels that returned to normal after symptomatic treatment.
§ Increased more than three times normal.
¶ Parasitemia >100,000/μl or >2% of the red blood cells infected.
the facilities for the management of its complications and the time of starting treatment. In a study of strictly defined cerebral malaria at a provincial hospital in eastern Thailand, the mortality rate was 21%, even though adequate facilities were available. At a teaching hospital, the case fatality rate was even higher (80%) if patients had acute pulmonary insufficiency and disseminated intravascular coagulation, although those patients were also treated in the intensive care unit. The mortality rate of severe malaria or its complications might be reduced if treatment with a potent antimalarial drug and the proper intervention treatment were initiated early. There were no deaths in this study probably because only 18 cerebral malaria patients were included, and these patients and their other complications received prompt treatment.

Overall cure rates in the three groups were low and not different among the groups as a result of the inadequate radical curative action of artesunate given for five days, which is comparable to earlier experience with this drug. None- theless, the immediate impact of artesunate on the malaria parasite was clearly demonstrated by the fact that all patients in this study survived despite a high percentage of multigang involvement on admission.

The pathophysiology of severe falciparum malaria is not clearly understood. Cytokines, especially TNF-α, have been postulated to play an important role in the pathophysiology of severe malaria. In African children with cerebral malaria, circulating levels of TNF-α and some other cytokines were found to be greatly elevated and correlated with clinical severity and complications such as the incidence of neurologic sequelae. This association between high cytokine levels and severity suggests that TNF-α and other cytokines may play a key role in the pathophysiology of cerebral and other severe forms of falciparum malaria. The recent finding that

### Table 3

<table>
<thead>
<tr>
<th>Therapeutic responses of the patients*</th>
<th>Group I (n = 15)</th>
<th>Group II (n = 15)</th>
<th>Group III (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever clearance time (hr) (n = 45)†</td>
<td>86 (72)</td>
<td>73 (73)</td>
<td>105 (54)</td>
</tr>
<tr>
<td>Range</td>
<td>10–264</td>
<td>22–274</td>
<td>16–194</td>
</tr>
<tr>
<td>Fever clearance time (hr) (n = 36)‡</td>
<td>56 (41)</td>
<td>52 (33)</td>
<td>80 (40)</td>
</tr>
<tr>
<td>Range</td>
<td>10–136</td>
<td>22–106</td>
<td>16–144</td>
</tr>
<tr>
<td>Parasite clearance time (hr) (n = 45)†</td>
<td>59 (17)</td>
<td>61 (24)</td>
<td>58 (29)</td>
</tr>
<tr>
<td>Range</td>
<td>33–84</td>
<td>28–103</td>
<td>6–139</td>
</tr>
<tr>
<td>No. of patients who dropped out§</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. of patients with 28-day follow-up</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>No. of patients with recrudescence (RI)</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Day at recrudescence (range)</td>
<td>18–20</td>
<td>19–23</td>
<td>16–23</td>
</tr>
<tr>
<td>% cure rate (cured/evaluable patients × 100)</td>
<td>50 (5/10)</td>
<td>67 (6/9)</td>
<td>56 (5/9)</td>
</tr>
</tbody>
</table>

* Except where indicated, values are the mean (SD). For definitions of groups, see Table 1.
† Including all patients (n = 45); no significant difference between the groups (P > 0.05).
‡ Excluding patients with sepsis (n = 36); no significant difference between the groups (P > 0.05).
§ Patients excluded who did not complete the 28-day follow up or had taken pretreatment with antimalarial drugs or were given mefloquine after artesunate treatment.
children with a variant allele of the TNF-α promoter region gene had a five times greater risk of developing cerebral malaria, and a 10 times greater risk of dying of this complication, supports this hypothesis. Neutralization of the high proinflammatory cytokine levels might therefore improve the clinical outcome of severe malaria. However, results of two reported clinical trials were conflicting. In a pilot study of 41 children with cerebral malaria in the Gambia, three different doses of a murine monoclonal antibody directed against TNF were administered and resulted in a dose-dependent reduction in fever. Another study using a monoclonal antibody to TNF in 610 Gambian children with cerebral malaria revealed no effect on the survival rate.

In this study, pentoxifylline was used to inhibit TNF-α, rather than an antibody as in the Gambian trial. In the high-dose patients (Group III), the TNF levels were reduced significantly within 6 hr from the start of treatment, while it took 24 hr in the low-dose (Group II) and placebo (Group I) groups, which showed similar TNF profiles. Details of the TNF and IL-6 levels and profiles are in a companion paper. The results of our clinical trial of pentoxifylline revealed no evidence of benefit from the use of this drug for ancillary treatment of severe malaria. The clinical evidence relates to speed of recovery from coma, duration of hemodilatation, time to normalization of the increased liver enzyme levels, and the period of endotracheal intubation. One patient each in Group II and Group III deteriorated in consciousness early during treatment but then recovered. There were no serious adverse effects seen in the two groups treated with pentoxifylline. In a study of cerebral malaria in Africa, children using pentoxifylline together with quinine showed a beneficial effect of the drugs on reduction in the duration of coma without a significant difference in parasite clearance time or time before defervescence.

The possible reasons for not showing any benefit in our study when the theoretical arguments look so convincing are three-fold. First, the antimalarial treatment was based on artemesunate; a very potent antimalarial drug that resolved the clinical manifestations quickly. Second, artemesunate has a similar action as artemether, which has been shown to inhibit the production of TNF-α. Finally, the number of patients in our study was relatively small and the results need to be confirmed in a larger study with antimalarial drugs that have no effect on TNF-α production. Last but not least, we studied adult patients, not children. Nevertheless, our results contrast sharply with those of an Italian study which showed a beneficial effect of pentoxifylline in African children with cerebral malaria, even though we used the maximum effective dose of pentoxifylline. The Italian study used a dose of 10 mg/kg/day, which is equal to 0.42 mg/kg/hr or half the pentoxifylline dosage of the low-dose pentoxifylline treatment group and one-fourth the dosage of the high-dose pentoxifylline group in our study.

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