EFFECT OF PENTOXIFYLLINE ON CYTOKINE PATTERNS IN THE THERAPY OF COMPLICATED PLASMODIUM FALCIPARUM MALARIA

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Abstract. The effect of pentoxifylline (PTX) was tested for its capacity to modulate cytokine responses during therapy of severe Plasmodium falciparum malaria in a placebo-controlled, randomized study in 45 adult patients in Bangkok, Thailand. The patients received standard antimalarial treatment with artesunate (120 mg intravenously given immediately, then 60 mg every 12 hr for a total dose of 600 mg). The patients received either low-dose PTX (20 mg/kg/day, n = 15), high-dose PTX (40 mg/kg/day, n = 15), or placebo (n = 15) as continuous infusion for the first three days of antimalarial treatment. Tumor necrosis factor (TNF) and interleukin-6 (IL-6) plasma levels were markedly elevated in all patients prior to treatment. After 6 hr of high-dose PTX treatment, TNF and IL-6 levels significantly decreased while an increase in TNF and IL-6 levels was seen after 6 hr of low-dose PTX or placebo treatment (P < 0.01). After 12 and 24 hr of high-dose PTX infusion, TNF-receptor plasma concentrations were lower than in low-dose PTX- or placebo-treated patients (P < 0.01), whereas no differences between the groups with regard to IL-6 receptor levels were observed. We conclude that 40 mg/kg/day of PTX reduces plasma levels of TNF, IL-6, and TNF-receptor in patients with severe malaria. Whether this reduction improves clinical outcome remains to be determined.

Plasma levels of tumor necrosis factor (TNF) correlate with disease severity in Plasmodium falciparum malaria. Excessive levels predispose an individual to cerebral malaria and fatal outcome.1, 2 Tumor necrosis factor induces enhanced adhesion molecule expression on endothelial cells. Intercellular adhesion molecule-1 (ICAM-1) and E-selectin act together with vascular cell adhesion molecule-1, thrombospondin, and CD36 as receptors for infected erythrocytes in vitro models of P. falciparum infection.3-5 Another TNF-induced mechanism in complicated malaria is the abundant production of oxygen-derived free radicals by neutrophils and mononuclear phagocytes.6 There is evidence that TNF is a central regulator molecule that mediates malaria complications including cerebral malaria in humans:1, 2, 9 and mice.10 Circulating heat-stable parasite antigens seem to be important inducers of TNF production.11 Pentoxifylline (PTX; 3,7-diethyl-1-(5-oxo-hexyl)-xanthine), a methylxanthine, inhibits TNF synthesis via the inhibition of phosphodiesterase and the increase of intracellular cyclic adenosine monophosphate. It has also been shown to depress TNF production by macrophages at the transcription level and reduce TNF bioactivity in a dose-dependent manner.12 This compound could have a beneficial effect on complications where this cytokine plays a role, e.g., acute respiratory distress syndrome, renal failure, or cerebral malaria. Pentoxifylline also increases deformability of red blood cells thereby counteracting the decreased deformability of infected erythrocytes.13, 14 It also has an effect on rosette formation by inhibiting red blood cell aggregation.15 In addition, PTX ameliorates the neutrophil-driven exacerbation of the erythrocyte cytoadherence-dependent microvascular obstruction and thus anoxic stasis, microthrombosis, ischemia, infarction, and hemorrhage.14 A study in mice and two case reports in humans suggest a beneficial effect of PTX in the treatment of severe malaria.16-19 Recently, PTX was shown to reduce the coma duration in children with cerebral malaria.20

To evaluate the effect of PTX applied intravenously for the first 72 hr, in addition to plasmodicidal therapy of severe P. falciparum malaria, a randomized, placebo-controlled study was performed in Bangkok, Thailand. The clinical results of this study are described in the report of Looareesuwan and others.21

PATIENTS AND METHODS

Patients. The study was conducted at the intensive care unit of the Hospital for Tropical Diseases in Bangkok, Thailand. The criteria for the selection of patients and the clinical details have been described by Looareesuwan and others.21 All patients had severe P. falciparum malaria (World Health Organization [WHO] classification, 1990) with a Glasgow coma score of 5-11. Eighteen patients had cerebral malaria, nine patients had renal failure, eight patients had azotemia, 23 patients had jaundice, and seven patients required intubation. The study was approved by the Institutional Review Board of the Hospital for Tropical Diseases. Written informed consent for each patient was obtained in accordance with Title 21, Parts 50 and 56 of the U.S. Code of Federal Regulations.

Treatment. All patients received standard antimalarial treatment with artesunate (120 mg intravenously given immediately, then 60 mg every 12 hr for a total dose of 600 mg) and were randomized into three groups receiving additional treatment: 1) high-dose PTX, 1.67 mg/kg/hr for the first 72 hr of therapy; 2) low-dose PTX, 0.83 mg/kg/hr for the first 72 hr of therapy; and 3) placebo for the first 72 hr of therapy. Antimalarial treatment and PTX were started at the same time. The PTX or placebo were administered in a continuous, rate-controlled, intravenous 0.9% NaCl infusion.

Laboratory assessments. Blood samples were collected into tubes containing EDTA prior to injection and after initiation of treatment at 6, 12, 24 and 48 hr, and on days 7, 14, 21, and 28. The blood specimens were centrifuged, and
the plasma was separated and immediately frozen at −70°C. The samples were air-shipped on dry ice to the Department of Infectious Diseases, University Hospital of Vienna. Plasma concentrations of TNF, TNF-receptor 55kD (sTNF-R55kD), interleukin-6 (IL-6), and IL-6 receptor were determined by ELISAs (R & D Systems, Oxford, United Kingdom). In healthy controls, the mean ± SD plasma level of TNF was 4.5 ± 2.1 pg/ml, the mean ± SD plasma level of sTNF-R55kD was 1.0 ± 0.4 ng/ml, the mean ± SD plasma level of IL-6 was 3.2 ± 1.2 pg/ml, and the mean ± SD plasma level of IL-6 receptor was 11.2 ± 4.1 ng/ml.

Statistical analysis. Laboratory results were compared with local reference ranges and the number (proportion) of patients with abnormal results at each time period was calculated. The results of laboratory tests were compared using the Kruskal-Wallis test. If significance between groups was detected, comparisons were made between control and treated groups and between the two treatment groups using the Wilcoxon-rank sum test. Hypotheses were tested two-sided; all results with a P value < 0.05 were considered statistically significant. The statistical software used for these analyses was Excel® (Microsoft Corp., Redmond, WA).

RESULTS

Parasitemia and fever. The levels of parasitemia are shown in Table 1. The median (range) duration of parasitemia was not different between the groups: 60 (33–103) hr in the placebo group, 52 (28–94) hr in the low-dose group, 52 (28–94) hr, and 59 (35–139) hr in the high-dose group (P > 0.05 for all comparisons). Data on fever are provided in Table 2. Again, no differences between the groups were observed. In addition, no correlation between fever and/or parasitemia and cytokine concentrations was seen. Additional clinical data are described in the report of Looareesuwan and others.21

Plasma concentrations of TNF. The median (range) plasma levels of TNF in the placebo, PTX low-dose, and PTX high-dose-treated patients prior to treatment were significantly elevated compared with controls: 188 (12–501), 79 (14–281), and 83 (15–316) pg/ml, respectively. There was no significant difference in plasma concentrations prior to therapy. Plasma concentrations of TNF started to decrease in the PTX high-dose–treated patients 6 hr after initiation of treatment and were found to be significantly decreased 12 hr after the start of therapy. In contrast, plasma concentrations of placebo- or PTX low-dose–treated patients increased 6 hr after the start of the infusion. In these patients, plasma TNF concentrations decreased after 12 hr of therapy and were significantly decreased after 48 hr in the PTX low-dose and in the placebo group (P < 0.01 for both compared with the high-dose PTX). At day 7, however, no difference between the treatment groups was seen (Table 3).

Tumor necrosis factor receptor 55kD. The median (range) plasma levels of sTNF-R55kD in placebo-, PTX low-dose–, and PTX high-dose–treated patients prior to treatment were significantly elevated compared with healthy controls: 18.0 (3.1–10), 8.1 (2.6–10), and 7.9 (4.1–10) ng/ml, respectively (Table 4). There was no significant difference in plasma concentrations prior to therapy. Plasma concentrations of sTNF-R55kD started to decrease in the PTX high-dose–treated patients even 6 hr after the initiation of treatment. In contrast, in placebo- or PTX low-dose–treated patients, plasma concentrations of sTNF-R55kD increased 6 hr after start of the infusion and decreased after 48 hr in the PTX low-dose treatment group and after 96 hr in the placebo group (P < 0.01 for both compared with the PTX high-dose group). At day 7, however, no difference between the treatment groups was seen.

Interleukin-6. The median (range) plasma levels of IL-6 in placebo-, PTX low-dose–, and PTX high-dose–treated patients prior to treatment were significantly elevated compared with healthy controls: 1,290 (20–500), 271 (19–500), and 285 (40–500) pg/ml, respectively. There was no significant difference in plasma concentrations prior to therapy. Plasma concentrations of IL-6 started to decrease in the PTX high-dose–treated patients 6 hr after the initiation of treatment and were found to be decreased 12 hr after the start of therapy. In contrast, in placebo-treated patients, plasma concentrations of IL-6 increased significantly 6 hr after start of the infusion. In these patients, plasma IL-6 concentrations

TABLE 1

<table>
<thead>
<tr>
<th>Parastemia</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>233 (34–1,056)</td>
<td>118† (10–921)</td>
<td>73† (3–886)</td>
<td>2† (0–376)</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>PTX, 20 mg/kg/day</td>
<td>160 (0–518)</td>
<td>125† (0–522)</td>
<td>387 (0–490)</td>
<td>0.3† (0–301)</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>PTX, 40 mg/kg/day</td>
<td>161 (10–861)</td>
<td>139† (3–847)</td>
<td>333 (3–793)</td>
<td>0.9† (0.2–288)</td>
<td>0.01</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* PTX = pentoxifylline.
† Significant difference between sequential time points within each treatment group; no differences between the groups existed.

<table>
<thead>
<tr>
<th>Fever</th>
<th>Temperature prior to therapy (°C)</th>
<th>Duration of fever (hr)</th>
<th>Days with fever prior to therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>38.2 (37.2–40.5)</td>
<td>56 (12–264)</td>
<td>4 (2–8)</td>
</tr>
<tr>
<td>PTX, 20 mg/kg/day</td>
<td>38.5 (37–40)</td>
<td>40 (10–274)</td>
<td>4 (2–10)</td>
</tr>
<tr>
<td>PTX, 40 mg/kg/day</td>
<td>38 (36.5–40)</td>
<td>91 (16–194)</td>
<td>4 (2–7)</td>
</tr>
</tbody>
</table>

* PTX = pentoxifylline.
decreased after 12 hr of therapy and were significantly decreased after 48 hr. In the PTX low-dose–treated patients, plasma IL-6 concentrations increased after 6 hr (P = 0.057; not significant), decreased significantly from 6 to 12 hr after therapy, and were reduced compared with baseline levels after 48 hr. At day 7, no difference between the treatment groups was seen (Table 5).

**Interleukin-6 receptor (IL-6R).** The median (range) plasma levels of IL-6R in placebo-, PTX low-dose–, and PTX high-dose–treated patients prior to treatment were significantly elevated compared with healthy controls: 58 (18–83), 43 (20–61), and 41 (30–90) ng/ml, respectively. There was no significant difference in plasma concentrations prior to therapy. In addition, no differences between the groups during therapy were seen since plasma concentrations of IL-6R did not decrease in all three groups of patients (Table 6).

**DISCUSSION**

Impaired perfusion due to sequestrated infected erythrocytes after TNF-mediated endothelial cell inflammation is thought to result in malaria complications such as cerebral malaria.1,22 Pentoxifylline is a methylxanthine that improves perfusion of peripheral and cerebral vascular tissue. In addition, an overall improvement in hemorheologic characteristics such as erythrocyte deformability, blood viscosity, platelet aggregation, and plasma fibrinogen concentrations has been described.14 It was also shown that PTX inhibits the inflammatory action of IL-1 and TNF on neutrophil function in terms of reducing the generation of free oxygen radicals and therefore reducing the neutrophil-mediated injury on endothelial cells.

Pentoxifylline was tested for its capacity to prevent cerebral malaria in *P. berghei* ANKA–infected CBA/Ca mice. Nine of 12 control mice developed neurologic signs and died of cerebral malaria about two weeks after infection. None of the 12 mice treated with PTX (1 mg) for 10 days developed cerebral malaria. However, all surviving mice developed high parasitemias and severe anemia and died two weeks later without neurologic signs. In PTX-treated mice, plasma TNF was not detectable, whereas control mice had high TNF levels on day 6 after injection. These findings were supported by in vitro investigations of malaria antigen–induced TNF synthesis. Anecdotal reports have suggested a beneficial effect in severe malaria.16,18,19

In the present study, after 6 hr of high-dose PTX treatment, TNF and IL-6 levels decreased significantly. However, 6 hr after low-dose PTX or placebo treatment, an increase in TNF and IL-6 levels was seen (P < 0.01). After 12 and 24 hr of high-dose PTX infusion, TNF-receptor plasma concentrations were lower than in low-dose PTX– or placebo-treated patients (P < 0.05), whereas no differences in IL-6 receptor levels between the groups were observed. In contrast, in quinine-treated children with cerebral malaria in Africa, PTX at a dose as low as 10 mg/kg/day also resulted in decreased TNF levels. In that study,20 the patients had higher TNF levels than in the current study. However, direct comparison is difficult since only 10 patients in the PTX-treated group and seven patients in the placebo group were tested for TNF and different analytic systems were used. Nevertheless, the better outcome in children (reduction in coma duration) in the PTX-treated patients was associated with reduced TNF levels on day 3.20 However, in our study, no beneficial clinical effects were observed.21 This discrepancy could be related to 1) differences in antimalarial therapy (artesunate therapy is known to result in more rapid parasite clearance than quinine treatment), 2) the age of the patients (adults in our study and children in the previous

### Table 3
Median (range) plasma levels of tumor necrosis factor (TNF) in the individual groups*

<table>
<thead>
<tr>
<th>Hours</th>
<th>Placebo (ng/ml)</th>
<th>PTX, 20 mg/kg/day (ng/ml)</th>
<th>PTX, 40 mg/kg/day (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (12–501)</td>
<td>6 (25–544)</td>
<td>12 (28–378)</td>
</tr>
<tr>
<td></td>
<td>87 (18–249)</td>
<td>24 (14–196)</td>
<td>48 (14–196)</td>
</tr>
<tr>
<td></td>
<td>25 (0–165)</td>
<td>48 (6–165)</td>
<td>96 (6–165)</td>
</tr>
</tbody>
</table>

*Significant difference between sequential time points within each treatment group.
†Significant difference between PTX, 20 mg/kg/day and controls and PTX, 20 mg/kg/day, respectively.

### Table 4
Mean (range) plasma levels of tumor necrosis factor (TNF)-receptor (ng/ml) in the individual groups*

<table>
<thead>
<tr>
<th>Hours</th>
<th>Placebo (ng/ml)</th>
<th>PTX, 20 mg/kg/day (ng/ml)</th>
<th>PTX, 40 mg/kg/day (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (3.1–10)</td>
<td>6 (4.6–10)</td>
<td>12 (4.8–10)</td>
</tr>
<tr>
<td></td>
<td>8.6 (4.8–10)</td>
<td>24 (4.8–10)</td>
<td>48 (2.7–10)</td>
</tr>
<tr>
<td></td>
<td>7.6 (1.7–10)</td>
<td>96 (1.7–10)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference between sequential time points within each treatment group.
†Significant difference between PTX, 20 mg/kg/day and controls and PTX, 20 mg/kg/day, respectively.
one), and 3) differences in the disease severity (although all patients confirmed the WHO criteria for severe malaria, not all patients had cerebral malaria in our study in contrast to the study by di Perri and others.\textsuperscript{20} In our study, significant differences in cytokine levels were seen only during the first 24 hr after initiation of therapy. Since the duration of disease was up to several days prior to admission, PTX may have to be administered as a first-aid outpatient treatment immediately after the establishment of the diagnosis.

In conclusion, we have demonstrated a faster plasma TNF and IL-6 concentration normalization in high-dose PTX-treated patients. We do not know whether these effects of PTX are mediated entirely by the suppression of TNF production. The array of various pharmacodynamic effects of PTX such as its effects on eicosanoid synthesis, blood viscosity, direct effects on adhesion molecule expression, and/or increased oxygen supply could also account for the results. Nevertheless, the findings of this study demonstrate that PTX therapy is able to reduce plasma TNF levels. No effect on the duration of parasitemia and duration of fever was seen. In contrast with a similar study in children,\textsuperscript{20} the effect of TNF suppression in our study failed to correlate with clinical efficacy.\textsuperscript{21} It remains to be elucidated whether PTX therapy is advantageous in selected quinine-treated adult patients with cerebral malaria with a poor clinical response.

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Tables 5 and 6 provide the median (range) plasma levels of interleukin-6 (IL-6) and IL-6 receptor (ng/ml) in the individual groups, respectively.

### Table 5

<table>
<thead>
<tr>
<th>IL-6 (pg/ml)</th>
<th>0 (20–500)</th>
<th>6 (30–500)</th>
<th>12 (28–500)</th>
<th>24 (20–500)</th>
<th>48 (0–175)</th>
<th>96 (0–19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>290</td>
<td>312</td>
<td>260*</td>
<td>89*</td>
<td>18*</td>
<td>0*</td>
</tr>
<tr>
<td>PTX, 20 mg/kg/day</td>
<td>271</td>
<td>359</td>
<td>262*</td>
<td>86*</td>
<td>17*</td>
<td>3*</td>
</tr>
<tr>
<td>PTX, 40 mg/kg/day</td>
<td>285</td>
<td>136†</td>
<td>125†</td>
<td>54‡</td>
<td>22‡</td>
<td>5†</td>
</tr>
</tbody>
</table>

\* PTX = pentoxifylline.
† Significant difference between PTX, 20 mg/kg/day and controls and PTX, 20 mg/kg/day, respectively.

### Table 6

<table>
<thead>
<tr>
<th>IL-6 receptor</th>
<th>0 (20–61)</th>
<th>6 (30–86)</th>
<th>12 (35–76)</th>
<th>24 (39–106)</th>
<th>48 (29–106)</th>
<th>96 (25–89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>58 (18–83)</td>
<td>52 (24–78)</td>
<td>49 (21–83)</td>
<td>60 (32–84)</td>
<td>57 (32–97)</td>
<td>54 (42–93)</td>
</tr>
<tr>
<td>PTX, 20 mg/kg/day</td>
<td>43 (20–61)</td>
<td>40 (30–86)</td>
<td>41 (35–76)</td>
<td>46 (29–106)</td>
<td>52 (41–79)</td>
<td>55 (25–89)</td>
</tr>
<tr>
<td>PTX, 40 mg/kg/day</td>
<td>41 (30–90)</td>
<td>47 (26–78)</td>
<td>49 (32–98)</td>
<td>49 (28–93)</td>
<td>56 (37–98)</td>
<td>54 (40–110)</td>
</tr>
</tbody>
</table>

\* PTX = pentoxifylline.

### References

10. Thumwood CM, 1989. Antioxidants can prevent cerebral ma-
PENTOXIFYLLINE FOR MALARIA