THE EFFECTS OF QUININE AND ARTESUNATE TREATMENT ON PLASMA TUMOR NECROSIS FACTOR LEVELS IN MALARIA-INFECTED PATIENTS

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Abstract. Tumor necrosis factor-alpha (TNF-α) is an endogenous mediator of shock and inflammation including malaria. Many lines of evidence suggest that cytoadherence, the life-threatening pathology associated with complicated and cerebral malaria, results from the overproduction of TNF in response to malarial parasite. Quinine has been shown to inhibit TNF synthesis and cytoadherence *in vitro* suggesting an additional beneficial effect of quinine on its anti-TNF action. On the other hand, artesunate inhibits cytoadherence better than quinine does not suppress TNF production *in vitro*. The present study compares the effect of artesunate and quinine on TNF levels of malaria-infected patients. Surprisingly, plasma TNF levels increased dramatically after quinine administration but did not increase after artesunate administration. This difference may be explained by previous observations showing that artesunate kills parasites *in vitro* and clears parasitemias *in vivo* for more rapidly than quinine. The rapid clearance of plasma TNF in quinine treated patients might be due to the drug's TNF-supressive activity.

INTRODUCTION

The development of drug resistant malaria is making efforts to control this disease more and more difficult. More than two million children die of cerebral malaria annually (Marsh *et al,* 1996). The mechanism of life-threatening malaria involves cytoadherence of parasitized erythrocytes in microvascular beds (Warrell, 1997). A crucial mediator of neurovascular lesions appears to be tumor necrosis factor-alpha (TNF-α) (Kremsner *et al,* 1995). TNF increases cytoadherence of infected erythrocytes to the brain vascular endothelium (Richards, 1997). Recently, it was shown that artesunate, artemether as well as quinine could inhibit both cytoadherence and rosetting (Udomsangpetch *et al,* 1996). Also, it was previously demonstrated that quinine could suppress the production of TNF in a dose-dependent manner *in vitro* (Picot *et al,* 1993; Kwiatkowski *et al,* 1995). Nevertheless, no evidence was found for inhibition of TNF response by artemether and artesunate (Kwaitkowski *et al,* 1995). Thus, the inhibitory effect of cytoadherence of quinine (Udomsangpetch *et al,* 1996) corresponds to its TNF suppression (Picot *et al,* 1993; Kwiatkowski *et al,* 1995). Artesunate inhibits cytoadherence more potently than quinine (Udomsangpetch *et al,* 1996) but by another mechanism. The present study compares the time-courses of plasma TNF levels in *Plasmodium falciparum*-infected patients after treatment with either artesunate or quinine.

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MATERIALS AND METHODS

Chemicals

Unless specified, all chemicals used in the present study were purchased from Sigma (St Louis, MO, USA).

Blood samples

Heparinized blood was collected from 12 patients infected with malaria. Six cases were uncomplicated, 5 cases were severe and 1 case was cerebral malaria. Two cases of severe and 1 case of uncomplicated malaria were treated with intravenous quinine (10 mg/kg body weight, 8 hourly). Another 1 case of cerebral, 3 cases of severe and 5 cases of uncomplicated malaria were treated with intravenous artesunate (2 mg/kg body weight). Blood was collected at various time intervals after drug administration from 0 minute to 24 hours. Fresh plasma was rapidly separated and freezed at -70°C until the TNF assay was carried out.

TNF assay

Quantitation of TNF levels was performed by

enzyme immunoassay kit (Predicta Tumor Necrosis Factor-α Kit, Genzyme, Cambridge, MA, USA). The microtiter plate was pre-coated with monoclonal antibody to TNF-α. Samples, standards or controls were added to each well and incubated to allow TNF to bind with antibody. A biotin-labeled polyclonal antibody to TNF was added and then followed by the addition of a peroxidase labeled streptavidin reagent. The color reaction was detected after the addition of peroxide and chromogen (tetramethyl benzidine). A standard curve was constructed to quantitate TNF-α concentrations in the controls and samples.

Statistics

Standard errors and theMann-Whitney U test were performed using SPSS for Windows Release 7.0 SPSS, Inc, Chicago, IL, USA.

RESULTS

The TNF plasma levels at various time intervals (0 minute - 24 hours) were determined from patients infected with malaria after administration of artesunate or quinine. The patients were allocated into 3 clinical presentations, uncomplicated malaria (UM), severe malaria (SM) and cerebral malaria (CM). We found no difference of TNF levels at all time points among these clinical presentations (data not shown). Also, there was no correlation between parasitemia, parasite clearance time and plasma TNF concentrations (Tables 1, 2).

Table 1 shows the clinical and parasitologic findings on admission of all 12 patients after intravenous administration with quinine or artesunate. There were no difference in baseline characteristics between these two groups.

As shown in Table 2 and Fig 1, the initial plasma TNF levels of the two groups were not significantly different ($p = 0.37$). The cytokine level was dramatically incresed and then slowly decreased after quinine administration. The peak concentrations during 30 minutes - 4 hours after drug administration (1,068 ± 68.6 to 1,319 ± 390 pg/ml) was 4.3 - 5.4 fold of the initial level. The plasma TNF then slowly decreased to 5.8 - 7.1 fold of the peak level after drug administration for 24 hours.

While a significant change of TNF levels was seen during quinine treatment, there was no change in TNF levels after artesunate administration. The cytokine levels at all time point except at 0 minute were higher after quinine than artesunate injection ($p < 0.05$).

DISCUSSION

Malaria pathology depends partly on parasite multiplication and partly on some elements of the immunological response (Richards, 1997). It has recently become evident that tumor necrosis factor (TNF) is directly implicated in the pathogenesis of cerebral malaria (Turner, 1997). TNF increases cytoadherence of parasitized erythrocytes to the brain microvascular endothelium (Richard, 1997). Soluble and heat-stable antigens from *Plasmodium falciparum* can induce TNF secretion by human macrophage *in vitro* (Jacobsen *et al*, 1995). This direct relationship between parasite and TNF secretion corresponded with the *in vivo* findings that TNF levels were significantly higher in malaria-infected patients than in other infections (Kwiatkowski *et al*, 1989).

The present study found no significant difference of plasma TNF concentration between uncomplicated, severe and cerebral malaria (data not shown). Also there was no correlation between the cytokine level and parasitemia as well as parasite clearance time. The results were agree with the previous reports (Grau *et al*, 1989; Baptista *et al*, 1997). Low TNF plasma concentrations have been found in severe malaria and high levels in mild cases (Grau *et al*, 1989). Recently, it was shown that there was no statistically significant difference of TNF level between cerebral and non-cerebral malaria. Also, there was no correlation between TNF level and clinical presentation, body temperature and parasitemia (Baptista *et al*, 1997).

However, other studies demonstrated significantly higher TNF levels in severe than uncomplicated

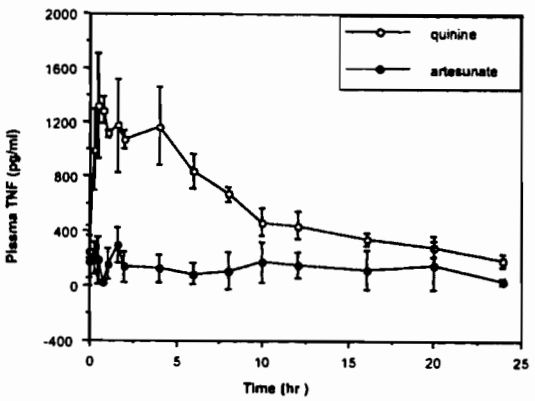

Fig 1–Plasma TNF level of malaria infected patients at various time points after administration with quinine and artesunate.

Table 1
Baseline clinical and parasitologic data on admission of 12 patients infected with malaria.

Clinical presentation*	Age (years)	Sex	Treatment (iv)	Parasitemia (%)	Parasite clearance (hours)**
UM	40	M	Quinine	17	NR
SM	50	M	Quinine	21	66
SM	53	M	Quinine	16	108
UM	19	M	Artesunate	14	NR
UM	38	M	Artesunate	6	90
UM	48	F	Artesunate	4	42
UM	26	M	Artesunate	2	>60
UM	20	M	Artesunate	1	30
SM	23	M	Artesunate	13	66
SM	20	M	Artesunate	3	66
SM	24	M	Artesunate	16	78
CM	20	F	Artesunate	8	54

* UM = Uncomplicated malaria, SM = Severe malaria, CM = Cerebral malaria
** NR = No record

Table 2
Plasma TNF concentrations at various time intervals of malaria-infected patients after intravenous administration with quinine and artesunate.

Time (hours)	TNF (pg/ml) after quinine, iv	TNF (pg/ml) after artesunate, iv
0	246 ± 185	182 ± 180
0.25	994 ± 298	201 ± 114
0.5	1,319 ± 390	184 ± 169
0.75	1,288 ± 97.6	25 ± 9
1	1,112 ± 26.2	158 ± 122
1.57	1,176 ± 347	294 ± 129
2	1,068 ± 68.6	136 ± 114
4	1,170 ± 284	125 ± 100
6	837 ± 127	84 ± 77
8	666 ± 55	110 ± 137
10	463 ± 104	171 ± 146
12	439 ± 103	151 ± 96
16	341 ± 42	119 ± 143
20	284 ± 76	157 ± 116
24	185 ± 47	33 ± 21

malaria (Kremaner *et al,* 1995; Medana *et al,* 1997). The contradictory results could be explained by the possibility of self-medication in endemic area (Picot *et al,* 1997) where chloroquine and other antimalarials are widely available over the counter. This chloroquine or quinine intake may modify the *in vivo* plasma TNF levels (Picot *et al,* 1993; Kwiatkowski *et al,* 1995). Also, the discrepancies could be a result of poor validity and reproducibility of TNF measurements (de Kossodo *et al,* 1995).

Experimentally, chloroquine and quinine can modulate TNF response in malaria-infected patients (Picot *et al,* 1993; Kwiatkowski *et al,* 1995). More than 60% of TNF was inhibited at the physiologic concentration of chloroquine while quinine showed 20-40% inhibition. It is possible that quinine, similar to chloroquine, inhibits production of TNF at the step of processing of membrane-bound pro-TNF to make soluble mature protein (Jeong and Jue, 1997). No evidence was found for inhibition of TNF re-

sponse by other drugs including artemether and artesunate (Kwiatkowski *et al,* 1995). These *in vitro* findings are consistent with the present *in vivo* findings, in that it is possible that the suppression of TNF by quinine only occurs after 6 hours.

Quinine inhibits cytoadherence *in vivo* and *in vitro* (Udomsangpetch *et al,* 1996) which could be a result of TNF supression. Suppression of cytoadherence by artemisinin derivatives, on the other hand, is not associated with TNF supression (Picot *et al,* 1993; Kwiatkowski *et al,* 1995).

Despite the *in vitro* effects of quinine of TNF, treatment of malaria with quinine was associated with a large surge in TNF production. TNF levels only fell to pretreatment levels after around 10 hours, suggesting that the TNF inhibitory effect was time-dependent. In contrast, artesunate treatment caused no increase in TNF levels, possibly because of its rapid onset of action. If TNF levels increase transiently after quinine administration, then it is possible that there could be a transient exacerbation of cerebral malaria. Such a TNF-mediated transient exacerbation would not occur after artesunate treatment. Further studies are needed to define the importance of this phenomenon clinically.

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