Ibuprofen does not affect levels of tumor necrosis factor α and soluble tumor necrosis factor receptor types I and II in Gabonese children with uncomplicated *Plasmodium falciparum* malaria

Pierre-Blaise Matsiegui[1,2], Michel A. Missinou[1,2], Saadou Issifou[1], Magdalena Necek[1,3], Elie Mavoungou[1,2]

[1] Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon
[2] Department of Parasitology, Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany
[3] Department of Internal Medicine, Division of Infectious Diseases, General Hospital of Vienna, Vienna, Austria

Correspondence : E. Mavoungou
<elie.mavoungou@uni-tuebingen.de>
Accepted for publication October 15, 2007

ABSTRACT. We assessed the ability of ibuprofen to modulate tumor necrosis factor α (TNF-α), soluble tumor necrosis factor receptor type I (sTNFR-I), and soluble tumor necrosis factor receptor type II (sTNFR-II) responses during the treatment of fever in uncomplicated *Plasmodium falciparum* malaria, in a placebo-controlled, randomized, double-blind study of 50 pediatric patients in Lambaréné, Gabon. Treatment of the malaria involved the patients receiving intravenous quinine (12 mg/kg of quinine dihydrochloride every 12 h for 72 h) followed by a single dose of oral sulfadoxine/pyrimethamine (25 mg and 1.25 mg/kg). Fever was treated by mechanical treatment plus either ibuprofen (7 mg/kg every 8 h) or placebo during the hospitalization period. We determined serum concentrations of TNF-α, sTNFR-I, and sTNFR-II in peripheral blood throughout the treatment period in the two groups: ibuprofen and placebo groups. TNF-α levels were found to be positively correlated with body temperature. In contrast, TNF receptors levels did not differ between the two groups and the antipyretic effect of ibuprofen was not correlated with specific changes in sTNFR-I and sTNFR-II production. Our data suggest that TNF-α is involved in malarial fever, but soluble TNF receptors play no major role in fever modulation.

Keywords: malaria, *falciparum*, TNF, fever, ibuprofen

Falciparum malaria is one of the leading killers of children in endemic areas [1]. The immunological processes leading to the clinical tolerance of plasmodium infection are poorly understood. Increases in tumor necrosis factor α (TNF-α) levels are often reported in patients with malaria, and TNF-α is involved both in malaria control and in malaria severity [2-6]. TNF-α has been shown to be correlated with parasitemia [3, 6], fever [7, 8], cerebral malaria [9-13], high levels of malaria-associated morbidity and mortality [4, 5] and poor infant outcome [14, 15]. Circulating heat-stable parasite antigens seem to be the principal inducers of TNF production [16]. The truncated extracellular domains of the two surface receptors (p55TNF-R and p75TNF-R) of TNF-α have been identified as TNF-α inhibitors [17]. These two domains are generated by proteolytic cleavage of the cell-surface tumor necrosis factor receptor (TNF-R) into soluble forms, referred to as soluble tumor necrosis factor receptor type I (sTNF-RI) and type II (sTNF-RII). Competitive sTNF-R/TNF-α complex formation and the intricate interactions between the activity and control of TNF-α [18, 19] in many diseases, including malaria, suggest that this process may also be important in malaria treatment. The role of peripheral blood TNF-α and its soluble receptors in the treatment of malaria, in the presence or absence of antipyretics, has never been investigated. To answer the question, "does the antipyretic effect of ibuprofen affect TNF production?", we evaluated the impact of treatment with ibuprofen — a non-steroidal anti-inflammatory drug — in addition to malaria therapy, for uncomplicated *P. falciparum* malaria. The clinical results of this study have been reported elsewhere [20]. We present here data concerning the role played by the antipyretic effect of ibuprofen on peripheral blood TNF-α, sTNFR-I and TNFR-II in children suffering from malaria. We compared children receiving antimalarial treatment plus ibuprofen to those receiving antimalarial plus placebo.

PATIENTS AND METHODS

Study site and participants

We conducted a randomized, double blind, placebo-controlled study between April 2003 and January 2004 at the Medical Research Unit of the Albert Schweitzer Hospital (MRU) in Lambaréné Gabon, where *P. falciparum* is

doi: 10.1684/ecn.2007.0105

hyperendemic, with a perennial mode of transmission and little seasonal fluctuation [21]. The entomological inoculation rate is about 50 infective bites per person per year and the main vectors are *Anopheles gambiae* and *Anopheles moucheti* [22]. Fifty children with uncomplicated *P. falciparum* malaria were recruited at the pediatric ward of the Albert Schweitzer Hospital. Informed consent was obtained from the parents or legal guardians of the children before their inclusion. The study was approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné and performed in accordance with the guidelines for human experimentation and clinical research of the Ministry of Public Health and Population of Gabon.

Blood collection

We collected 1 mL of venous blood every eight hours into a sterile, heparin-containing tube. Blood samples were immediately centrifuged for 10 minutes at 400 g in a Biofuge Pico centrifuge (Heraeus Instruments), to separate the pellets containing the packed erythrocytes from the plasma. Plasma samples were then frozen and stored at -80°C until analysis.

Malaria diagnosis

Two experienced laboratory microscopists checked for the presence of *P. falciparum,* asexual, blood-stage parasites in Giemsa-stained, thick blood smears, according to standard quality-controlled procedures. Parasite load was determined and expressed as the number of asexual forms of *P. falciparum*/µL of blood [23].

Treatment

For the malaria treatment, all patients received an infusion of 250 mL of 5% glucose with 12 mg/kg of quinine dihydrochloride every 12 h for 72 h, followed by a single dose of oral sulfadoxine/pyrimethamine (25 mg and 1.25 mg/kg). For the treatment of fever, all patients received mechanical treatment (continuous fanning and cooling blanket), when the body temperature rose above 37.5°C. In addition to the mechanical treatment, patients were assigned randomly to receive ibuprofen syrup (7 mg/kg) or placebo, every eight hours for the duration of the hospitalisation. The children were hospitalized until two consecutive, thick blood smears were negative for asexual malaria parasites and fever clearance.

Cytokine determinations

Commercially-available, human-specific ELISA kits (Alexis Biochemicals) were used in accordance with the manufacturer's instructions to determine the concentrations of TNF-α, sTNFR-I, and sTNFR-II in serum samples frozen at -80°C and were tested in duplicate. Materials supplied by the manufacturer were used to produce a standard curve. Absorbance was read on a densitometer (Multiskan R EX, Thermo, Vantaa, Finland) at 450 nm and plotted against a calibration curve with values in pg/mL for TNF-α or ng/mL for sTNFR-I and sTNFR-II.

Statistical analysis

Data were analyzed with StataCorp 2001 (Stata Statistical Software, Release 9.2; Stata Corporation, College Station, TX, USA). We recorded demographic, clinical and laboratory data for the patients on a case report form, entered into EXCEL. Data were cleaned before analysis. Differences between groups were assessed using Chi-squared or Fisher's exact tests for proportions and Student's *t*-test or the non-parametric Kruskal-Wallis test (for non-normal data) to compare continuous variables. Spearman's rank correlation test was used to assess correlations between continuous variables. P-values less than 0.05 were considered to be significant in two-tailed tests.

RESULTS

Baseline characteristics of study patients

From the 1230 children who were prescreened for malaria, 430 had positive blood smear, 50 met the inclusion criteria and were randomly assigned to treatment and analyzed. *table 1* lists the baseline characteristics of the patients. The two groups were similar with respect to baseline characteristics. The mean age of the patients was four years, mean temperature was 38.4°C and median parasite load was 62 250 parasites/µL.

Plasma TNF-α, sTNF-RI and sTNF-RII concentrations

Table 2 summarizes the findings concerning the measurements of the levels of TNF-α, sTNF-RI and sTNF-RII. We compared plasma TNF-α concentrations between the two groups of children on admission (0 hour) and 8, 16, 24 and 48 hours after the beginning of the antipyretic treatment. We found conflicting results. The median TNF-α concentration in the children on placebo was higher than that in children who received ibuprofen after 16 hours (33.11 *versus* 20.57) and after 24 hours (126.55 *versus* 121.62), but the difference was not significant. However, the median TNF-α concentration was higher in children taking ibuprofen than in children taking placebo at other time points (131.30 *versus* 124.21 at 0 hour, 27.26 *versus* 14.64 at 8 hours, and 127.9 *versus* 111. 98 at 48 hours). This difference was also not significant.
Measurements of sTNF-RI and sTNF-RII also showed no statistically significant difference between the two groups at different time point.

Effects of antipyretic treatment on plasma cytokine concentrations

Median plasma cytokine concentrations did not differ between the two groups (ibuprofen *versus* placebo) at the start of treatment or on day 2. However, major differences in cytokine levels between individuals were observed. Various approaches (i.e. analysis of variance, linear mixed model) were used to analyze the effect of the treatments, but no significant differences between the two groups were found in terms of the magnitude of change in TNF-α, sTNF-RI or sTNF-RII concentrations over time, or the number of individuals for whom plasma cytokine concentrations increased or decreased between time points.

Table 1
Baseline characteristics of children with *P. falciparum* infection

	Total	Ibuprofen	Placebo	p
Number of children	50	25	25	-
Median age in years	4	4	5	NS[a]
Gender (F/M)	18/32	10/15	8/17	NS
Mean temperature (°C)	38.4	38.1	38.7	NS
[SD]		1.1	1.0	1.0
Median parasite density	62 250	546 000	678 000	NS
[range]	[20 000-200 000]	[20 000-200 000]	[20 000-200 000]	
Median hemoglobin (g/dL) [range]	9.7 [7.1]	9.6 [7.1-10.1]	9.7 [7.1-11.8]	NS
Median hematocrit % [range]	29.8 [20.9-38.3]	29.9 [21.0-38.3]	29.7 [20.9-37.9]	NS

[a] Non-significant.

Correlation of cytokine concentrations with time, temperature and parasitemia

The data for all children were pooled for correlation analysis, as no significant differences in cytokine levels were observed between the groups. From the start of the study until day 3, a significant positive correlation was found between TNF-α concentration and body temperature ($p = 0.007$).

DISCUSSION

TNF is a cytokine with the potential to cause serious harm if produced in excessively large amounts or over a prolonged period. In this randomized, placebo-controlled study in Gabonese children, high circulating TNF-α concentrations at inclusion were found to be associated with episodes of malarial fever [4, 24]. This association remained significant after correction for parasitemia and was not affected by plasma concentrations of the soluble TNF receptors, sTNF-RI and sTNF-RII. These data are consistent with previous studies showing that TNF mediates malarial fever, but are not consistent with the hypothesis that inhibitory TNF-binding proteins play an important role in clinical tolerance in African children with malaria. We found that these high circulating TNF-α concentrations decreased during the course of malaria treatment, regardless of whether the child was given ibuprofen or placebo. Soluble TNF receptor levels did not differ significantly between children treated with ibuprofen and those given placebo. This brings us back to our initial study question:

Table 2
Plasma cytokine levels evaluated at different times in the placebo group (n = 25) and the ibuprofen group (n = 22)[$] of Gabonese children during uncomplicated *P. falciparum* malaria infection

Cytokines	Drugs	0 hours	8 hours	16 hours	24 hours	48 hours
TNF-α*	Ibuprofen	131.30	27.261	20.57	121.62	127.9
		[6.05-332.71]	[5.99-217.28]	[10.91-293.2]	[8.27-266.04]	[7.98-301.85]
		NS[&]	NS	NS	NS	NS
	Placebo	124.21	14.04	33.11	126.55	111.98
		[7.11-326.0]	[6.72-306.79]	[7.8-261-72]	[6.48-292.59]	[6.044-295.67]
TNF-RI[§]	Ibuprofen	3.667	4.134	3.497	3.383	2.929
		[2.44-30]	[1.85-21.42]	[1.95-23.99]	[2.05-23.54]	[2.14-31.41]
		NS	NS	NS	NS	NS
	Placebo	5.803	4.488	3.535	3.976	4.444
		[4.39-24.55]	[2.81-29.35]	[2.53-22.65]	[2.93-10.06]	[1.83-19.53]
TNF-RII[§]	Ibuprofen	34.935	21.42	32.50	23.14	26.14
		[7.46-50.22]	[5.07-51.36]	[4.69-56.41]	[6.087-51.94]	[11.01-56.28]
		NS	NS	NS	NS	NS
	Placebo	26.98	20.37	18.48	15.82	18.87
		[8.41-45.47]	[5.62-41-66]	[5.22-30.25]	[10.06-43.2]	[10.02-40.76]

[&] Non-significant; Wilcoxon/Kruskal-Wallis tests (rank sums); * pg/mL, median [interquartile range]; [§] ng/mL, median [interquartile range]; [$] two children in the ibuprofen group were excluded for protocol violation (one for axillary temperature measurement, one for consecutive missing temperature values for 24 hours). One patient had convulsions 12 hours after the start of the antipyretic and quinine treatment, and was excluded from the study.

"do the antipyretic effects of ibuprofen affect TNF production?" Ibuprofen is thought to function by inhibiting cyclooxygenase (i.e. COX-1 and COX-2), thereby inhibiting prostaglandin synthesis. The antipyretic and anti-inflammatory activities of ibuprofen seem to be mediated principally by COX-2 inhibition [25]. In malaria patients, soluble TNF receptor levels and immunoreactive TNF concentrations are strongly correlated [26, 27]. These receptor molecules are effective TNF antagonists, due to their ability to form complexes with the ligand [28, 29], and receptor constructs have therefore been used in immunotherapy [30]. High soluble receptor levels may also reflect the extensive cleavage of membrane-associated receptors, leading to a decrease in the number of binding sites potentially mediating TNF activity [31, 32]. However, TNF receptors have been shown to be involved in cerebral malaria, whereas the children studied here all had uncomplicated malaria. Strong protection against cerebral malaria has been demonstrated in TNF-RII$^{-/-}$ mice but not in TNF-RI$^{+/+}$ mice, and this protection has been shown to be associated with a lack of ICAM-1 upregulation [33]. A study on children from Ghana showed protection to be strongly correlated with serum concentrations of TNF-α and soluble TNF-α receptors only in patients with uncomplicated malaria. However, despite the association between TNF-α concentration and fever, in this study in Ghana, no differences in soluble TNF receptor levels were observed [9]. In the present study, we detected sTNF-RI and sTNF-RII in the ng/mL range, corresponding to a 100- to 1000-fold molar excess over TNF-α levels in children with malaria. Our results suggest that TNF-inducing activity *in vivo* may have been affected by antimalarial treatment, whether or not an antipyretic was also taken. It is also possible that the ability of the parasite to induce TNF is influenced by intrinsic host factors, including the acute inflammatory response in particular as antipyretic treatment had no effect on fever clearance in children with malaria [34].

In conclusion, our data show that there is an association between fever and plasma TNF-α concentration but demonstrate that the antipyretic effect of ibuprofen cannot be accounted for by changes in the production of this cytokine. Further studies are required to identify the stage in the inflammatory process at which ibuprofen exerts its principal anti-inflammatory and anti-pyretic effects.

Acknowledgments. *We thank all the children who participated in the study. We also thank Anselme Ndzengue, Drs Sunny Oyakhirome and Akim Adégnika for their help. Many thanks to Dr. Lipp and his group from University of Tübingen, Germany for the supply of ibuprofen and placebo. We are grateful to Eric Kendjo for statistical analysis and Brigitte Migombet, Ariane Ntseyi and Marcel Nkeyi for technical assistance. This work was supported by a grant to EM from the UNICEF/United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases (grant A50073).. Informed consent was obtained from all participants and the study was carried out in accordance with the guidelines for human experimentation in clinical research published by the Gabonese Ministry of Public Health and Population. Ethical clearance was obtained by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné.. Financial support: Prof. Elie Mavoungou is the recipient of a fellowship from the Alexander von Humboldt Foundation (N° 7000166012). This work was supported by a grant to EM from the UNICEF/United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases (grant A50073).*

REFERENCES

1. Munday S. Review of intermittent preventive treatment for malaria in infants and children. *J Paediatr Child Health* 2007; 43: 424.
2. Kern P, Hemmer CJ, Damme JV, *et al.* Elevated tumor necrosis factor alpha and interleukin-6 serum levels as markers for complicated *Plasmodium falciparum* malaria. *Am J Med* 1989; 87: 139.
3. Kern P, Hemmer CJ, Gallati H, *et al.* Soluble tumor necrosis factor receptors correlate with parasitemia and disease severity in human malaria. *J Infect Dis* 1992; 166: 930.
4. Kremsner PG, Winkler S, Brandts C, *et al.* Predictions of accelerated cure in Plasmodium falciparum malaria by the elevated capacity of tumor necrosis factor production. *Am J Trop Med Hyg* 1995; 53: 532.
5. Richards AL. Tumor necrosis factor and associated cytokines in the host's response to malaria. *Int J Parasitol* 1997; 27: 1251.
6. Day NP, Hien TT, Schollaardt T, *et al.* The prognostic and pathophysiologic role of pro- and anti-inflammatory cytokines in severe malaria. *J Infect Dis* 1999; 180: 1288.
7. Mordmüller BG, Metzger WG, Juillard P, *et al.* Tumor necrosis factor in *Plasmodium falciparum* malaria: high plasma level is associated with fever, but high production capacity is associated with rapid fever clearance. *Eur Cytokine Netw* 1997; 8: 29.
8. McGuire W, D'Alessandro U, Stephens S, *et al.* Levels of tumour necrosis factor and soluble TNF receptors during malaria fever episodes in the community. *Trans R Soc Trop Med Hyg* 1998; 92: 50.
9. Akanmori BD, Kurtzhals JA, Goka BQ, *et al.* Distinct patterns of cytokine regulation in discrete clinical forms of *Plasmodium falciparum* malaria. *Eur Cytokine Netw* 2000; 11: 113.
10. Gimenez F, Barraud de Lagerie S, *et al.* Tumor necrosis factor alpha in the pathogenesis of cerebral malaria. *Cell Mol Life Sci* 2003; 60: 1623.
11. Lucas R, Juillard P, Decoster E, *et al.* Crucial role of tumor necrosis factor (TNF) receptor 2 and membrane-bound TNF in experimental cerebral malaria. *Eur J Immunol* 1997; 27: 1719.
12. Molyneux ME, Engelmann H, Taylor TE, *et al.* Circulating plasma receptors for tumour necrosis factor in Malawian children with severe *falciparum* malaria. *Cytokine* 1993; 5: 604.
13. Piguet PF, Kan CD, Vesin C. Role of the tumor necrosis factor receptor 2 (TNFR2) in cerebral malaria in mice. *Lab Invest* 2002; 82: 1155.
14. Fried M, Muga RO, Misore AO, *et al.* Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes. *J Immunol* 1998; 160: 2523.
15. Moorman AM, Sullivan AD, Rochford RA, *et al.* Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation. *J Infect Dis* 1999; 180: 1987.
16. Taverne J. Two soluble antigens of *Plasmodium falciparum* induce tumor necrosis factor release from macrophages. *Infect Immun* 1990; 58: 2923.
17. Engelmann H, Aderka D, Rubinstein M, *et al.* A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. *J Biol Chem* 1989; 264: 11974.
18. Aderka D. The potential biological and clinical significance of the soluble tumor necrosis factor receptors. *Cytokine Growth Factor Rev* 1996; 7: 231.
19. Carpentier I, Coornaert B, Beyaert R. Function and regulation of tumor necrosis factor type 2. *Curr Med Chem* 2004; 11: 2205.

20. Matsiegui PB, Missinou MA, Necek M, et al. Antipyretic effect of ibuprofen in Gabonese children with uncomplicated falciparum malaria: a randomized, double blind, placebo controlled trial. (Submitted).
21. Wildling E, Winkler S, Kremsner PG, *et al.* Malaria epidemiology in the province of the Moyen Ogooué, Gabon. *Trop Med Parasitol* 1995; 46: 77.
22. Sylla EH, Kun JF, Kremsner PG. Mosquito distribution and entomological inoculation rates in three malaria-endemic areas in Gabon. *Trans R Soc Trop Med Hyg* 2000; 94: 652.
23. Planche T, Krishna S, Kombila M, *et al.* Comparison of methods for the rapid laboratory assessment of children with malaria. *Am J Trop Med Hyg* 2001; 65: 599.
24. Brandts CH, Ndjavé M, Graninger W, *et al.* Effect of paracetamol on parasite clearance time in *Plasmodium falciparum* malaria. *Lancet* 1997; 350: 1776.
25. Renda G, Tacconelli S, Capone ML, *et al.* Celecoxib, ibuprofen and antiplatelet effect of aspirin in patients with osteoartnritis and ischemic heart disease. *Clin Pharmacol Ther* 2006; 80: 264.
26. Kern P, Hemmer CJ, Gallati H, *et al.* Soluble tumor necrosis factor receptors correlate with parasitemia and disease severity in human malaria. *J Infect Dis* 1992; 166: 930.
27. Molyneux ME, Engelmann H, Taylor TE, *et al.* Circulating plasma receptors for tumor necrosis factor in Malawian children with severe falciparum malaria. *Cytokine* 1993; 5: 604.
28. Engelberts I, Stephens S, Francot GJM, *et al.* Evidence for different effects of soluble TNF-receptors on various TNF measurements in human biological fluids. *Lancet* 1991; 338: 515.
29. Lesslauer W, Tabuchi H, Gentz R, *et al.* Recombinant soluble tumor necrosis factor receptor proteins protect mice from lipopolysaccharide-induced lethality. *Eur J Immunol* 1991; 21: 2883.
30. Ashkenazi A, Marsters SA, Capon DJ, *et al.* Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesin. *Proc Natl Acad Sci USA* 1991; 88: 10535.
31. Van Zee KJ, Kohno T, Fisher E, *et al.* Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* 1992; 89: 4845.
32. Schleiffenbaum B, Olgiati L, Fehr J. TNF-specific deactivation of granulocytes *in vivo* a possible mechanism of self protection. *Eur J Haematol* 1992; 49: 239.
33. Medana IM, Hunt NH, Chaudhri G. Tumor necrosis factor- expression in the brain during fatal murine cerebral malaria: evidence for production by microglia and astrocytes. *Am J Pathol* 1997; 150: 1473.
34. Lell B, Sovric M, Schmid D, Luckner D, *et al.* Effect of antipyretic drugs in children with malaria. *Clin Infect Dis* 2001; 32: 838.