

tions of Alaska, and for reversed elucidation of potential genetic resistance markers indicating metacestode abortion [4].

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References

- Gottstein B. *Echinococcus multilocularis* infection: immunology and immunodiagnosis. *Adv Parasitol* 1992;31:321-80.
- Ewald D, Eckert J, Gottstein B, Straub M, Nigg H. Parasitological and serological studies on the prevalence of *Echinococcus multilocularis* Leuckart, 1863 in red foxes (*Vulpes vulpes* Linnaeus, 1758) in Switzerland. *Rev Sci Tech* 1992;11:1057-61.
- World Health Organization. Report of the WHO informal consultation on *Echinococcus multilocularis* research. Geneva: WHO 1988.
- Rausch RL, Wilson JF, Schantz PM, McMahon BJ. Spontaneous death of *Echinococcus multilocularis*: cases diagnosed serologically by Em2-ELISA and clinical significance. *Am J Trop Med Hyg* 1987;36:576-85.
- Liance M, Vuitton D, Guerret-Stocker S, Carbillet JP, Grimaud JA, Houin R. Experimental alveolar echinococcosis. Suitability of a murine model of intrahepatic infection by *Echinococcus multilocularis* for immunological studies. *Experientia* 1984;40:1436-9.
- Kroeze WK, Tanner CE. *Echinococcus multilocularis*: susceptibility and responses to infection in inbred mice. *Int J Parasitol* 1987;17:873-83.
- Gottstein B, Mesarina B, Tanner I, et al. Specific cellular and humoral immune responses in patients with different long-term courses of alveolar echinococcosis (infection with *Echinococcus multilocularis*). *Am J Trop Med Hyg* 1991;45:734-42.
- Tiercy JM, Jeannet M, Mach B. A new approach for the analysis of HLA class II polymorphism: HLA oligotyping. *Blood Rev* 1990;4:9-15.
- Bettens F, Pichler WJ, de Weck AL. Incorporation of biotinylated nucleotides for the quantification of PCR-amplified HIV-1 DNA by chemiluminescence. *Eur J Chem Clin Biochem* 1991;29:685-8.
- Doherty DG, Vaughan RW, Donaldson PT, Mowatt AP. HLA DQA, DQB and DRB genotyping by oligonucleotide analysis: distribution of alleles and haplotypes in British caucasoids. *Hum Immunol* 1992;34:53-63.

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Nitric Oxide in Cerebral Malaria

Colleagues—Cerebral malaria is an important complication of *Plasmodium falciparum* infection and a major cause of infant mortality in Africa. The pathophysiology of cerebral malaria remains incompletely understood. Recently, a link between cytokines, nitric oxide (NO), and cerebral malaria was proposed [1, 2]. It was hypothesized that intravascular NO, induced by tumor necrosis factor in endothelial cells and vascular smooth muscle, diffuses through the blood-brain barrier and interferes with neurologic function. Furthermore, NO is thought to increase intracranial pressure, a common feature in cerebral malaria in African children [3], through increased vasodilatation. Our data do not support this concept.

We studied the evolution of NO plasma concentrations in 28 African children (mean age \pm SD, 4.4 \pm 3 years) with cerebral malaria in Yaoundé. All children presented with unrousable coma and *P. falciparum* parasitemia. Coma depth was assessed according to the modified Glasgow coma scale for young children [4]. Other causes of coma were excluded. After treatment with quinine (25 mg of base/kg daily for 8 days), the clinical outcome was favorable in 21 cases; 4 children developed neurologic sequelae. Three children died: after 12 h, 3 days, and 4 days. Mean duration of coma for the 25 surviving children was 45.1 \pm 31.4 h. NO plasma concentrations were measured on the day of admission (day 1) and on days 2, 3, 5, and 8 (discharge). Stable NO end products (NO₂⁻ and NO₃⁻) were measured by means of an automated procedure, as described previously [5].

On day 1, mean NO concentration in 28 children was 44.3 \pm

36.5 μ M. Children with favorable outcome presented with a higher mean NO concentration (49.9 \pm 40.3 μ M) than those with neurologic sequelae (30.6 \pm 15.5 μ M) or those who died (23.7 \pm 4.6 μ M). NO concentrations were higher in children with a coma score of 3 ($n = 11$; 63.2 \pm 49 μ M) than in children with a coma score of 1 or 2 ($n = 17$; 32.2 \pm 18.6 μ M). These differences were not statistically significant. NO concentrations were negatively correlated with the duration of coma (Spearman rank test, $r = -.49$, $P < .02$), indicating that the duration of coma was significantly longer in children with NO plasma levels $< 44.3 \mu$ M than in those with NO plasma levels superior to the mean (53.6 \pm 27.5 vs. 32.4 \pm 34.0 h; $P < .02$). No significant correlation between NO level and platelet or white blood cell counts, blood glucose level, parasitemia, hemoglobin level, or body temperature was found.

We observed an increase of mean NO concentration after 24 h of quinine treatment in children with favorable outcome (group A); in children with sequelae (group B), this was found after 48 h. By day 3, mean NO level was higher in group A (44.2 \pm 50.9 μ M) than in group B (12.8 \pm 3.9 μ M) (Mann-Whitney U test, $P = .03$). Similar results were observed at day 5 (62.1 \pm 68.1 vs. 16.2 \pm 7.1 μ M; $P < .04$; figure 1).

Although NO plasma concentrations are higher during cerebral malaria than during uncomplicated malaria attacks (unpublished data), our results seem to refute the hypothesis that increased NO levels are detrimental in the development of cerebral malaria. In a murine model, *N*-monomethyl-*L*-arginine, an inhibitor of NO synthase, prevented neither the development of neurologic symptoms nor the death of *Plasmodium berghei*-infected mice [6, 7]. Furthermore, treatment with a competitive NO synthase inhibitor had a deleterious effect in complicated murine *Plasmodium vinckei* malaria [8]. Therefore, it has been suggested that NO has a protective role in cerebral malaria [9]. Further investigations are being done to confirm the protective effect of NO in human pathology as suggested by murine models and by our preliminary results.

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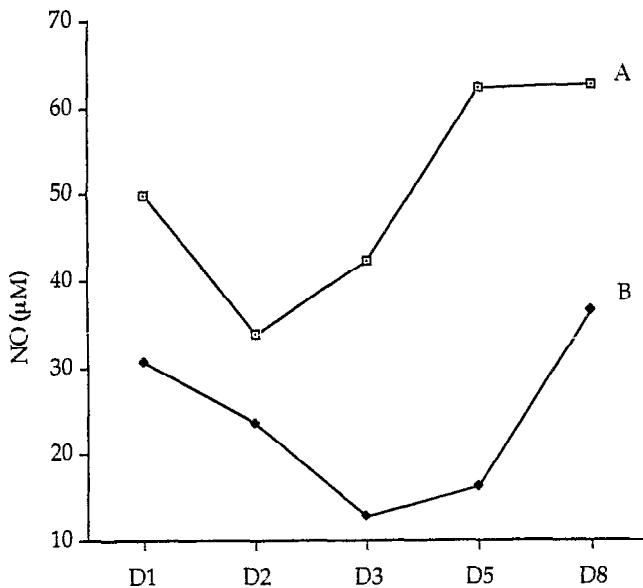


Figure 1. NO plasma concentrations in children with favorable outcome (group A, $n = 21$) and children with neurologic sequelae (group B, $n = 4$). D = day.

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Patients with *Plasmodium falciparum* Malaria and *Plasmodium vivax* Malaria Show Increased Nitrite and Nitrate Plasma Levels

Colleagues—The most severe complication of *Plasmodium falciparum* malaria is the cerebral syndrome, which accounts for >1 million deaths every year. It has been proposed that nitric oxide accounts, at least in part, for the pathophysiology seen in human cerebral malaria [1]. Nitric oxide, a ubiquitously occurring molecule, is released from several cells in response to cytokines, including tumor necrosis factor- α [2], which is associated with cerebral malaria [3]. Several in vivo studies in humans show that nitric oxide forms during sepsis or immunotherapy [2]. However, it is unclear whether nitric oxide is produced in

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References

- Clark IA, Rockett KA, Cowden WB. Proposed link between cytokines, nitric oxide and human cerebral malaria. *Parasitol Today* 1991;7:205-7.
- Rockett KA, Awburn MM, Aggarwal BB, Cowden WB, Clark IA. In vivo induction of nitrite and nitrate by tumor necrosis factor, lymphotoxin, and interleukin-1: possible roles in malaria. *Infect Immun* 1992;60:3725-30.
- Newton CRJC, Kirkham FJ, Winstanley PA, et al. Intracranial pressure in African children with cerebral malaria. *Lancet* 1991;337:573-6.
- Molyneux ME, Taylor TE, Wirima JJ, Borgstein J. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose children. *Q J Med* 1989;71:441-59.
- Ochoa JB, Udekwo AO, Billiar TR, et al. Nitrogen oxide levels in patients after trauma and during sepsis. *Ann Surg* 1991;214:621-6.
- Asensio VC, Oshima H, Falanga PB. *Plasmodium berghei*: is nitric oxide involved in the pathogenesis of mouse cerebral malaria? *Exp Parasitol* 1993;77:111-7.
- Kremsner PG, Nüssler A, Neifer S, et al. Malaria antigen and cytokine-induced production of reactive nitrogen intermediates by murine macrophages: no relevance to the development of experimental cerebral malaria. *Immunology* 1993;78:286-90.
- Kremsner PG, Neifer S, Chaves MF, Rudolph R, Bienzle U. Interferon- γ induced lethality in the late phase of *Plasmodium vinckei* malaria despite effective parasite clearance by chloroquine. *Eur J Immunol* 1992;22:2873-8.
- Senaldi G, Kremsner PG, Grau GE. Nitric oxide and cerebral malaria. *Lancet* 1992;340:1554.

humans during malaria and whether increased nitric oxide formation is associated with the pathology seen in malaria.

We studied the nitric oxide plasma levels in patients infected with *P. falciparum* or *Plasmodium vivax* in Vietnam and Brazil. The Vietnam study included 34 patients with cerebral malaria (mean age 35 years, range 15-67) hospitalized in the Cho Ray Hospital of Ho Chi Minh City, 12 patients with uncomplicated malaria, and 4 controls. Nitric oxide plasma levels were determined on the day of admission by an automated procedure as described [4]. The median nitric oxide levels were as follows: controls, 23 μM (range, 15-51); patients with uncomplicated malaria, 40 μM (range, 12-93); and patients with cerebral symptoms, 71 μM (range, 16-487), which were significantly higher than levels in the other two groups (Kruskall Wallis test, $P < .01$). Eight patients with cerebral malaria died. Their nitric oxide levels (median 75, range 31-487) did not differ from those of survivors.

The Brazil study, done in an outpatient clinic in Rio Branco, Acre, included 15 healthy controls and 44 patients (age range, 16-50 years; mean, 26) with uncomplicated *P. falciparum* malaria. Twenty-eight patients were nonimmune, and 16 were classified as semiimmune [5]. A third group consisted of 61 patients (age range, 15-60 years; mean, 28) with *P. vivax* malaria. Nitric oxide levels determined on admission were as follows: healthy

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