N-ACETYLCYSTEINE IN SEVERE FALCIPARUM MALARIA IN THAILAND

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Abstract. One hundred and eight patients with severe falciparum malaria underwent a placebo controlled trial with the antioxidant, N-acetylcysteine (NAC), as an adjunctive therapy along with standard intravenous artesunate therapy. Three NAC dosage regimens were used: an intravenous loading dose of 140 mg/kg followed by 70 mg/kg every four hours intravenously for up to 18 doses (Group 1); a single intravenous loading dose followed by oral NAC in the same amount as for Group 1 (Group 2); a regimen identical to Group 1 except that oral NAC was administered after the first 24 hours (Group 3). Fifty-four patients received placebo plus artesunate. Two critically ill patients died in Group 1. No patient sustained an adverse reaction to the NAC other than vomiting, and the deaths were attributed to severe disease with multiple organ involvement. The excellent results with NAC, the lack of adverse effects, and the rationale for NAC benefit supports the need for a large, double blind trial of NAC as an adjunctive therapy for severe malaria.

INTRODUCTION

Because of the high mortality of severe falciparum malaria and its rapidly changing spectrum of responsiveness to therapeutic agents, there is a need for new ways to treat the disease. It would be ideal for a new method to attack the basic pathophysiology of the disease and thereby circumvent the problem of developing drug resistance of the malarial organism. To meet this challenge, a number of adjunctive therapies have been proposed for severe malaria, including dexamethasone, hyperimmune sera, low molecular weight dextran, heparin, tumor necrotic factors, antibody therapy, trental treatment, and surfactant. Only phenobarbital and partial exchange transfusion have been shown to be effective and then not uniformly so (Newton and Krishna, 1998). The search for new adjunctive therapies should be based on current understanding of the basic molecular aspects of severe malaria.

Many facets of severe malaria involve the activation of cytokines (Nyakundi *et al*, 1994; Udomsangpetch, *et al*, 1997), endothelial surface adhesion molecules (Jakobsen *et al*, 1994; Brown *et al*, 2000), and nitric oxide synthase (Maneerat *et al*, 2000). All these mediators have in common that they are at least partially activated by the transcription factor, nuclear factor-κB (NF-κB) (Carroll, 2000). We have demonstrated that the powerful antioxidant N-acetylcysteine (NAC) inhibits activation of NF-κB (Hess *et al*, 1994). In an experimental model of stroke reperfusion injury, which has many similarities to the molecular pathophysiology of severe malaria, we also demonstrated that NAC reduces tissue injury (Carroll *et al*, 1998). NAC is now widely used for the treatment of acetaminophen toxicity (Perry and Shannon, 1998; Yip *et al*, 1998). The rationale for NAC is further strengthened by the obser-

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vation of Thumwood and co-workers (1989) that antioxidants prevented cerebral malaria in a mouse model.

Therefore, we conducted a trial of NAC in 54 patients with severe falciparum malaria, diagnosed according to the WHO (2000) criteria, and admitted to the Bangkok Hospital of Tropical Diseases (BHTD). Fifty-four patients received placebo. The purpose of this trial was to determine the relative safety and tolerability of NAC in this population and to learn if the drug might have sufficient potential for efficacy to justify a large double-blind, controlled trial. A secondary purpose was to determine the most workable method of administration for patients with severe malaria.

PATIENTS AND METHODS

Patients with severe malaria patients admitted to BHTD between March 2001 and April 2002 were enrolled into the study if they were 13 years or older. Patients were considered to have severe falciparum malaria (WHO, 2000) if they had unarousable coma (Warrell *et al*, 1982), renal impairment (serum creatinine concentration > 265 μmol/l (3 g/dl) after rehydration), jaundice (serum bilirubin concentration > 50 μmol/l (3 mg/dl)), hyperparasitemia (greater than 100,000 parasites/μl), the presence of schizonts, or severe anemia (hemoglobin <5 g/dl). The patients agreed to remain in the hospital for 28 days to assess the late adverse effects and watch for late recrudescence. Informed consent was obtained from the patient or relatives. Patients were excluded if they were pregnant or lactating. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Once a patient fulfilled the inclusion criteria, they were randomized with a sealed, coded envelope. The envelope was then opened and the patient assigned to the proper group accordingly. The clinicians were blinded as to the treatment. All patients received standard treatment for severe falciparum malaria with intravenous artesunate followed by oral artesunate for a total dose of 600 mg in 5 days and followed by oral mefloquine (25 mg/kg) given in two divided doses 8 hours apart. In the case of recrudescence, other antimalarials (standard doses of quinine plus tetracycline for 7 days) were used.

Three separate protocols were utilized for NAC administration. In the first protocol, a 3% solution of NAC with 5% dextrose was given as an intravenous loading dose of 140 mg/kg over one hour, with subsequent doses of 70 mg/kg every four hours over one hour each for a total of up to eighteen doses (Group 1). In the second protocol, NAC was first given as an intravenous loading dose and then the route of administration was switched to oral NAC when patients appeared well enough to tolerate oral medication (Group 2). In the third protocol, the regimen was the same as Group 1 except that NAC was administered intravenously for 24 hours only followed by oral NAC as in group 2 (Group 3).

Additionally, 54 patients were managed as above except that 0.9 % saline placebo was administered rather than NAC (Group 4).

All patients were hospitalized for 28 days to assess safety, tolerability, and the possibility of recrudescence of infection. Body temperature, pulse, and respiration rate were evaluated every 4 hours during the study. Blood pressure was measured every 6 hours for 7 days and then once daily while patients stayed in the hospital. The patients' signs and symptoms were evaluated daily for the first seven days and weekly thereafter, including a neurologic examination focused on brain stem and cerebellar functions, muscle strength in all limbs, extraocular and facial muscle strength, deep tendon reflexes, and finger-to-nose test. Side effects were defined as those signs or symptoms that first occurred or became worse after treatment was started. Cure was defined as the absence of recrudescence during the 28 days follow-up.

Before treatment was begun, all patients received red and white blood cell counts with differential, electrolytes, total and direct biliru-

bin, alkaline phosphatase, blood urea, creatinine, albumin, globulin, aspartate and alanine aminotransferases, and urine analysis. These were repeated as often as necessary for management while the patient was seriously ill and on days 7, 14, 21, and 28. Thick and thin blood films were prepared and examined for parasites every 6 hours during treatment until they were found to be negative, and then once a day during the 28 day follow-up. Malaria parasite count per microliter was obtained by calculation against the red blood cell count for a thin film or against the white blood cell count for a thick film. The films were considered negative if no parasites were seen in 200 oil-immersion microscopic fields. Parasite clearance time (PCT) was defined as the period from the start of treatment until the first negative blood film (Looareesuwan *et al*, 1992). Fever clearance time (FCT) was taken as the period from the start of treatment until the oral temperature decreased to 37.5ºC and remained below this temperature for the next 48 hours (Looareesuwan, *et al*, 1992).

We compared the characteristics of the patients who received NAC, including number with hyperparasitemia (>100,000/mm^3), those having cerebral malaria, jaundice, severe vomiting, presence of schizonts, anemia, or renal failure requiring hemodialysis, PCT and FCT, number with late recrudescence of the malarial parasite, number who dropped out of the study, and the number who died with these same factors among the patients who did not receive NAC.

RESULTS

A total of 108 patients with severe falciparum malaria were enrolled in the study, 31 in Group 1, 5 in Group 2, and 18 in Group 3 (54 total). Fifty-four patients were treated with the standard severe malaria protocol plus placebo for NAC (Group 4). Admission data are shown in Table 1. Because the patients did not tolerate the early administration of oral NAC, Group 2 was stopped after only five patients (due to large amount of liquid volume). The patients in Group 2 were older, but we did not assess this difference for significance because of the small size of the group. Sex and initial parasite counts were not significantly different. Medical events during the treatment are shown in Table 2. The groups of patients did not differ significantly for medical complications, degree of parasitemia, or other clinical or laboratory phenomena.

Outcome data are shown in Table 3. Two patients died in Group 1. The first was a seriously ill 31 year old male admitted to the Intensive Care Unit of BHTD in the evening because of hyperparasitemia (13.8%) and intractable vomiting. He developed cardiac arrest at 05.00 hour the next morning and could not be resuscitated. He had received only three

Table 1
Admission characteristics.

	Group 1	Group 2	Group 3	Group 4
Male/female	23/8	3/2	12/6	30/24
Age (years)				
Mean (SD)	32.7±12.7	45.2±18.2	26.1±9.7	24.8±11.7
Range	14-60	20-65	18-45	13-76
Initial parasite count				
Mean (SD)	280,833 ±288,205	209,392 ±174,375	156,302 ±233,958	249,993 ±244,277
Range	4,840-994,400	30,450 433,780	231-748,000	3,730-954,810

Table 2
Medical events.

	Group 1	Group 2	Group 3	Group 4
Cerebral malaria	0/31	0/5	0/18	0/54
Renal failure				
Requiring dialysis	5/31	0/5	1/18	2/54
Jaundice	13/31	2/5	9/18	25/54
Hyperparasitemia (>100,000 parasites/μl)	20/31	3/5	6/18	36/54
Presence of schizonts	22/31	5/5	16/18	38/54
Severe vomiting	16/31	5/5	10/18	35/54
Anemia	9/31	0/5	5/18	22/54
Elevated transaminases	10/31	0/5	3/18	8/54

Table 3
Outcomes.

	Group 1 (N=31)	Group 2 (N=5)	Group 3 (N=18)	Group 4 (N=54)
Fever clearance time (hours)	68.4±40.8	76±41.3	52.6±35.8	52.9±34.8
Parasite clearance time (hours)	58.3±16.8	62±2.7	53.4±26.9	52.1±16.8
Dropped out of study[a]	4/31	0/5	3/18	10/54
Late recrudescence	1/31	0/5	0/18	1/54
Died	2/31	0/5	0/18	0/54

[a]Dropped out of study: not related to adverse effect of NAC but due to social reasons (*eg*: desire to return home and unable to return for follow-up within 28 days). All patients were well with no fever or parasitemia before they withdrew from the study.
Data expressed as mean ± SD.

doses of NAC. The second patient who died was a 58 year old male who had been treated at two other hospitals for the same bout of malaria. He was critically ill in all of these facilities with peripheral cyanosis, jaundice, and hematuria. Upon admission to BHTD, he was anemic with elevated liver enzymes, and his bleeding time was prolonged. He underwent hemodialysis. After admission to BHTD he survived for two days and received eight doses of NAC.

In Group 1, four patients withdrew from the study due to their personal decisions. All were clear of parasitemia and fever at the time of their withdrawal. Another patient had a recurrence of parasitemia on day 17 and was successfully treated with quinine and tetracycline for 7 days. No other patients had recurrences.

In Group 2, none had a recurrence of parasitemia. Overall, these severely ill patients did not easily tolerate the oral administration of NAC. The drug was extremely unpalatable orally and often resulted in vomiting by this route. Consequently, the early oral route of administration was abandoned.

In Group 3, three patients dropped out of the study for personal reasons; all were clear of parasites and fever. None had a recurrence of parasitemia.

Among those treated with the standard protocol without NAC, ten dropped out due to personal reasons. Another patient developed a

recurrence of parasitemia on day 25. PCT and FCT were not statistically different among the four groups. Other than the lack of palatability of the NAC and the vomiting it induced among the Group 2 patients, no side effects of the NAC were observed. There were no allergic reactions to the drug in any of the patients.

DISCUSSION

Two of the 54 patients with severe malaria who received NAC died (3.7%). Survival rates among patients with severe malaria vary widely depending upon the population studied, complications, organ involvement and the treatment facility carrying out the study (Warrell *et al*, 1982). In any case, this death rate of 3.7% compares favorably with the best survival rates reported for severe malaria.

FCT and PCT did not differ between Groups 1, 2, and 3 and Group 4 (the latter group not treated with NAC). We would not expect NAC to have any effect on these variables, unless NAC somehow suppressed the individual's ability to mount a satisfactory immune response to the parasite. This could occur via suppression of the normal cytokine response (Newton and Krishna, 1998). If that had been the case, we might have observed a prolongation in PCT among the NAC-treated patients. This was not seen. No adverse effects of intravenous NAC were recognized in this population, and this route of administration was well-tolerated. Our impression was that oral administration of the drug would not be suitable early in the treatment of severe malaria.

NAC was chosen as an adjunctive treatment for malaria because many lines of research point to the theoretical potential of the drug in this disease process. Many aspects of severe malaria, such as elevated cytokines (Nyakundi *et al*, 1994; Udonsangpetch *et al*, 1997), intracellular adhesion molecules (Jakobsen *et al*, 1994; Brown *et al*, 2000) and nitric oxide synthase (Maneerat *et al*, 2000) indicate that activation of the transcription factor, NF-κB, may play a central role in the pathophysiology of the disease. NAC is an exquisite inhibitor of NF-κB (Hess *et al*, 1994) and a safe antioxidant. The latter group of drugs has been demonstrated as effective in an experimental model of malaria (Thumwood *et al*, 1989). On theoretical grounds, NAC should be most effective in patients with severe malaria, who have demonstrable abnormalities of the blood vessels, such as renal failure, adult respiratory distress syndrome or cerebral malaria. Indeed, the drug may not have much effect unless the patients are in such an extreme state. Thus, while other factors may be examined for the possible benefit of NAC, death rate would clearly be the best marker for efficacy of the drug. Alternatively, use of NAC might reduce the incidence of serious complications like cerebral malaria and renal failure.

Ideally, one would like to have a simple, safe, and effective adjunctive method of treating severe malaria, which could be used in basic medical care facilities. Adjunctive measures avoid the continuing problem of developing drug resistance. Transfer of patients to tertiary, well-equipped medical care facilities, like the BHTD, even though mortality rates are clearly lower in such facilities, is not practical for the large majority of severely ill patients. The restoration of body homeostasis, including volume repletion, normalization of pH, and attainment of normoglycemia, is the mainstay of adjunctive management. Currently, adjunctive methods such as exchange transfusion and the administration of phenobarbital for seizures remain confined to situations relying on clinical judgement. Higher doses of phenobarbital are associated with an unexpectedly higher mortality rate (Crawley *et al*, 2000) and there is no rationale for the use of phenobarbital in patients who do not have cerebral malaria. Obviously, exchange transfusion cannot be offered in most locations. Corticosteroids may be deleterious in severe malaria (Warrell *et al*, 1982).

We conclude that intravenous NAC is safe and well-tolerated in adult patients with severe malaria. The mortality rate overall among the NAC-treated patients was very low, suggesting

that the drug may be an effective adjunctive therapy in these patients. The scientific rationale for its use in severe malaria is robust and needs further study in a large, double-blind randomized clinical trial.

Therefore, a large scale trial of NAC seems warranted. NAC is relatively inexpensive and could be prepackaged for intravenous use for a 24 hour period, as we employed in Group 3. A trial of sufficient sample size and statistical power could then be conducted in outlying facilities where most patients with severe malaria are treated. While there are many variables which could be studied to assess efficacy, such as the complications of adult respiratory distress syndrome or renal failure, PCT and FCT, or various serum factors such as cytokines, the study should be directed toward improvement in mortality rates.

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