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# A pilot study of N-acetylcysteine as adjunctive therapy for severe malaria

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## Summary

**Background:** The case fatality rate of severe malaria remains unacceptably high. N-acetylcysteine (NAC) is a safe compound that inhibits tumour necrosis factor (TNF) and impedes cytoadherence, both of which have been implicated in the pathogenesis of malaria complications.

**Aim:** To evaluate NAC as adjunctive therapy in severe malaria.

**Design:** A placebo-controlled, double-blind prospective study, with serum lactate level as the principal objective measure of response.

**Methods:** Thirty adult males with severe, quinine-treated malaria received either 300 mg/kg of NAC or placebo, over 20 h.

**Results:** Serum lactate levels normalized twice as quickly after NAC (median 21 h, 95%CI 12–36 h)

as after placebo (median 42 h, 95%CI 30–84 h;  $p=0.002$ , Mann–Whitney U test). Twenty-four hours after admission, 10/15 (67%) NAC-group patients but only 3/15 (20%) placebo-group patients had normal lactate concentrations ( $p=0.01$ , Fisher exact test). NAC-treated patients could be switched from intravenous to oral therapy earlier than individuals who received placebo (42 h vs. 51 h after admission) but the difference was not significant ( $p=0.28$ , Mann–Whitney U test).

**Discussion:** NAC's mechanism of action in malaria is unclear, since it did not markedly alter plasma cytokine profiles. Trials of NAC adjunctive therapy for complicated malaria, with mortality as an endpoint, appear to be warranted.

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## Introduction

Treatment modalities capable of reducing the high case fatality rate of severe malaria are urgently needed.<sup>1</sup> Tumour necrosis factor (TNF) and other cytokines have been implicated in the pathogenesis of severe malaria.<sup>2</sup> Pro-inflammatory cytokines mediate cerebral dysfunction in murine cerebral malaria<sup>3</sup> and TNF- $\alpha$  mRNA is expressed in brain tissues in human cerebral malaria.<sup>4</sup> The case fatality rate of cerebral malaria was not reduced by anti-TNF monoclonal antibody,<sup>5</sup> but other options are available to inhibit cytokines. N-acetylcysteine (NAC) inhibits TNF release

and is a potent scavenger of free oxygen radicals,<sup>6</sup> which are produced in response to TNF and mediate some of its toxic effects. Unfortunately, there is no suitable animal model for testing NAC in severe human malaria, but the drug has accumulated an impressive safety during its many years of use in treating acetaminophen (Tylenol, Paracetamol) overdose.<sup>7–9</sup> We therefore performed a placebo-controlled, double-blind pilot study of adjuvant treatment with NAC for severe *Plasmodium falciparum* infection.

## Methods

### Patients and study design

Informed consent was obtained from patients or their guardians, and human experimentation guidelines of the Ethical Review Committee of the Thai Ministry of Public Health and of the Human Subjects Research Review Board, Office of the Surgeon General, Department of the United States Army were followed. Adult patients admitted to Maesod Hospital, western Thailand, with *Plasmodium falciparum* infection and no illness other than severe malaria were enrolled. Most patients with severe malaria seen at the study hospital are young males. We therefore limited enrolment to males aged 18–50 years, to maximize homogeneity between treatment groups. Malaria was severe if there was hyperparasitaemia ( $>100\,000$  parasites per  $\text{mm}^3/\text{blood}$ ), serum bilirubin  $>3.0$  mg/dl, renal dysfunction (urine output less than 16.7 ml/h or serum creatinine  $>3.0$  mg/dl), pulmonary oedema or adult respiratory distress syndrome, hypoglycaemia, shock, spontaneous bleeding, repeated generalized convulsions, or impaired consciousness (coma score  $<15$ , Glasgow coma scale).<sup>1</sup>

Endpoints were established before the study began. Treating physicians not involved in the study but responsible for patient care at Maesod Hospital decided whether or not dialysis was required. If a decision to dialyse was made, patients were transferred out of the ward where the study was conducted, and were not followed further by the study team. Patients were evaluated by the investigators and by study nurses every 6 h. If consciousness was normal (coma score = 15), there was neither nausea nor vomiting, and the patient stated that he was capable of taking oral medication, antimalarial drugs were given orally rather than intravenously. Decisions to switch to oral medication were made without knowledge of whether the patient had received NAC or placebo, and was one of the endpoints of the study. The study team no longer followed patients receiving oral quinine.

This investigation was designed as a 'first-look', pilot evaluation of NAC in 20 patients. We prospectively decided to analyse the serum lactate results after the first 20 patients, with three likely outcomes: (a) NAC clearly lowered serum lactate concentration; (b) NAC clearly did not lower serum lactate level; or (c) results were inconclusive because of the small sample size. If results were inconclusive, a further 10 patients were to be enrolled and randomized to receive either NAC or placebo. The interim analysis of serum lactate

levels from the first 20 study subjects showed that lactate concentration fell by a median of 0.3 pg/ml/patient in patients who received NAC and rose by a median of 0.3 pg/ml/patient in individuals given placebo ( $p=0.06$ , Mann–Whitney U test). This result suggested a possible lactate-lowering effect of NAC, but was not conclusive. NAC was well tolerated and therefore we decided to enrol a further 10 patients in the study. Five were randomized to receive NAC and five received placebo.

### Treatment

All patients received intravenous quinine dihydrochloride; administered as a loading dose of 20 mg/kg followed by maintenance doses of 10 mg/kg every 8 h.<sup>10</sup> Volunteers were assigned to receive either NAC or placebo according to a pre-determined computer-generated list of random numbers. Patients were assigned to receive either NAC or placebo as soon as a diagnosis of severe malaria was made, and both quinine and NAC (or placebo) were administered concurrently. Treatment was begun within 2 h of arrival at the study hospital. NAC was given as a loading dose of 150 mg/kg infused in 200 ml of 5% dextrose intravenously over 15 min, followed by 50 mg/kg in 500 ml of 5% dextrose over the next 4 h and 100 mg/kg in 1 l of 5% dextrose over the next 16 h. A total of 300 mg/kg of NAC were therefore administered over 20 h. Placebo was 5% dextrose in water, which is a colourless liquid identical in appearance to NAC. NAC and placebo solutions were made up by a designated nurse not otherwise involved in the study, and were administered by constant infusion. The addition of NAC to the 5% dextrose solution did not alter its appearance. Therefore neither physician nor patient was aware of whether the active or the placebo solution was being administered. Supportive therapy was given as necessary.

### Clinical and laboratory testing

Patients were examined for signs of severe malaria and venous lactate was measured (YSI 2300 glucose/lactate analyser) every 6 h until a study endpoint was reached. Glasgow coma scores were determined at 6-hourly intervals on patients with signs of neurological dysfunction on admission (coma score  $<15$ ). TNF- $\alpha$ , interferon- $\gamma$ , IL-6 and IL-10 were assayed by ELISA using commercially available kits (R&D Systems) every 6 h for the first 24 h after admission.

## Statistical analyses

Cytokine levels 24 h after admission were compared with baseline values using the Wilcoxon signed rank test. The Mann–Whitney U test was used to compare the duration of raised venous lactate, the duration of intravenous quinine therapy, and the duration of abnormal coma score in the NAC and placebo groups. The proportion of patients with normal serum lactate levels 24 h after admission were compared by Fisher's exact test. The Wilcoxon rank sum test was used to determine distribution-free confidence limits for median values. Multiple regression was used to illuminate the relationship between admission cytokine levels and changes in serum lactate during the 24 h after admission, and the effects of treatment group (NAC or placebo) and cytokine levels on changes in serum lactate were assessed by analysis of covariance. Two-sided testing was performed in all cases and  $p$  values  $<0.05$  were considered significant.

## Results

### Patients

Admission characteristics of the 15 patients randomized to receive NAC, and volunteers allocated to the placebo group, are shown in Table 1. Parasitaemia and IL-6 levels were higher in the placebo group, but differences were not significant ( $p=0.95$  and  $p=0.70$ , respectively). IL-10

concentrations were lower and CPK levels higher in NAC-treated individuals, but not significantly so ( $p=0.42$  and  $p=0.15$ , respectively). Most patients had more than one sign or symptom of severe malaria. Six individuals randomized to the NAC arm had hyperparasitaemia ( $>100\,000$  parasites per  $\text{mm}^3$ ), but in only one case was hyperparasitaemia the only criterion used to classify malaria as severe. Eight patients in the placebo arm had  $>100\,000$  parasites per  $\text{mm}^3$ , but none were classified as severe on the basis of high parasitaemia alone.

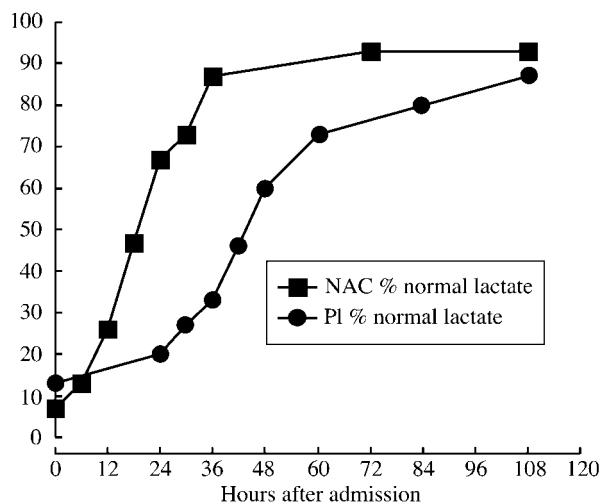
### Serum lactate (Figure 1)

Pre-treatment serum lactate levels were elevated ( $>2.5$  mmol/l) in 14 of 15 NAC-treated patients and in 13 of 15 patients who received placebo. Lactate concentrations returned to normal twice as quickly in NAC-treated individuals (median 21 h, 95%CI 12–36 h) as in subjects allocated to receive placebo (median 42 h, 95%CI 30–84 h;  $p=0.002$ , Figure 1). Significantly more NAC-treated patients had normal lactate levels at 24 h than did individuals who received placebo (10/15 vs. 3/15, respectively,  $p=0.011$ ). Serum lactate levels at 24 h were measured in 13 patients in the NAC arm and 14 patients in the placebo arm. Compared with pre-treatment concentrations, lactate fell by a median of 0.9 mmol/l (95%CI 0.1–2.0 mmol/l) at 24 h in the patients who received NAC. Lactate concentrations were higher at 24 h than on admission in subjects who received placebo (median rise 0.3 mmol/l, 95%CI  $-0.5$  to  $+2.1$  mmol/l).

**Table 1** Characteristics on admission for 30 adult male subjects with severe falciparum malaria

Treatment group	N-acetylcysteine ( $n=15$ )	Placebo ( $n=15$ )
Age (years)	28 ( $\pm 10$ )	27 ( $\pm 9$ )
History of previous malaria (yes/no)	8/6 (1 unknown)	5/9 (1 unknown)
Parasitaemia (/ $\text{mm}^3$ )	49 500 (43–885 500)	148 733 (114–858 000)
Days of fever prior to admission	5 (2–15)	5 (3–8)
Oral temperature	7 °C ( $\pm 0.8$ )	37.9 °C ( $\pm 1.2$ )
Glasgow coma score	14 (8–15)	12 (6–15)
Haematocrit	35% ( $\pm 9$ )	29% ( $\pm 7$ )
White blood cells/ $\text{mm}^3$	8140 (1540–15 400)	8195 (3410–22 000)
Platelets ( $\times 1000$ )/ $\text{mm}^3$	151 (45–503)	154 (75–270)
Serum lactate level (pg/ml)	3.1 (1.5–6.0)	2.9 (1.0–14.1)
Creatinine phosphokinase (U/l)	94 (20–1023)	41 (12–590)
Blood urea nitrogen (mg/dl)	23 (1–87)	18 (1–117)
Creatinine (mg/dl)	1.3 (0.7–4.2)	1.3 (0.7–8.9)
Total bilirubin (mg/dl)	0.8 (0.3–17.9)	1.8 (0.3–24.4)
Tumor necrosis factor- $\alpha$ (pg/ml)	33.4 (21.2–552.2)	34.2 (23.0–1261.0)
Interferon- $\gamma$ (pg/ml)	7.2 (0–285.4)	8.2 (0–276.7)
IL-6 (pg/ml)	20.2 (5.8–1369.0)	81.2 (7.7–1486.0)
IL-10 (pg/ml)	208.9 (4.4–1005.5)	565.5 (36.5–1045.0)

Values are mean ( $\pm$ SD) or median (range).



**Figure 1.** Proportion of patients (out of 15) with normal serum lactate concentration after administration of N-acetylcysteine (NAC) or placebo (PI). Note that at 24 h, serum lactate levels were normal in 67% of patients who received NAC (squares) but in only 20% of individuals who received placebo (circles).

The changes in lactate level during the 24 h after enrolment in the study were significantly different between NAC- and placebo-treated subjects ( $p=0.013$ , Mann-Whitney U test). The change in lactate concentration between 0 h and 24 h was not related to admission levels of TNF, interferon- $\gamma$ , IL-6 or IL-10 ( $p>0.1$ ) but was related to treatment ( $p=0.016$ , Fisher's PLSD).

### Patient outcome

No NAC-treated patient required dialysis, although one patient died 24 h after enrolment following a seizure. Three individuals randomized to receive placebo underwent dialysis, and one of these patients died. The death occurred in a subject admitted with impaired consciousness (coma score 8), hyperparasitaemia (183 920 parasites per  $\text{mm}^3$ ), and renal dysfunction (serum creatinine 4.8 mg/dl). Six hours after admission to the study, the patient suffered a cardiac arrest and was transferred to the intensive care unit. Twelve hours after admission, the patient died despite mechanical ventilation, circulatory support and dialysis. Two other patients in the placebo arm required dialysis, one 30 h and the other 72 h after enrolment.

### Duration of treatment with intravenous quinine

Intravenous quinine could be discontinued and oral therapy instituted after a median of 42 h in the 15 patients in the NAC group (95%CI 24–60 h) compared with 51 h in the 15 individuals allocated

**Table 2** Median (range) plasma cytokine concentrations during the first 24 h after admission

Treatment group	N-acetylcysteine	Placebo
<i>TNF-<math>\alpha</math> (pg/ml)</i>		
0 h	33 (21–552)	34 (23–1261)
6 h	29 (20–113)	38 (25–378)
12 h	29 (21–131)	36 (23–317)
18 h	28 (21–107)	35 (23–164)
24 h	27 (21–222)	30 (23–193)
<i>Interferon gamma (pg/ml)</i>		
0 h	7 (0–285)	8 (0–277)
6 h	10 (0–340)	14 (0–412)
12 h	4 (0–169)	6 (0–885)
18 h	7 (0–133)	5 (0–194)
24 h	9 (0–142)	5 (0–241)
<i>IL-6 (pg/ml)</i>		
0 h	20 (6–1369)	81 (8–1486)
6 h	19 (8–998)	85 (8–1313)
12 h	14 (8–900)	31 (7–1668)
18 h	12 (7–890)	36 (8–913)
24 h	12 (6–974)	31 (7–1110)
<i>IL-10 (pg/ml)</i>		
0 h	209 (4–1006)	566 (37–1045)
6 h	212 (11–839)	350 (20–931)
12 h	169 (6–840)	241 (19–975)
18 h	168 (3–821)	218 (15–952)
24 h	186 (3–918)	279 (9–903)

In the NAC arm, cytokine levels were measured in 15 patients at 0 h, 6 h, 12 h and 18 h and in 14 patients at 24 h. In the placebo arm, cytokine levels were measured in 15 patients at 0 h, 6 h, and 12 h and in 14 patients at 18 h and 24 h.

to the placebo group (95%CI 24 h–120 h,  $p=0.28$ , Mann-Whitney U test).

### Resolution of abnormal mental status

Seven NAC-treated volunteers and 10 patients who received placebo had coma scores less than 15 on admission. Coma score tended to become normal (score 15) more quickly in the NAC group (median 24 h, 95%CI 6 h–60 h) than in subjects given placebo (median 36 h, 95%CI 18 h–120 h), but the difference was not significant ( $p=0.19$ , Mann-Whitney U test).

### Cytokine concentrations (Table 2)

Compared with pre-treatment values, TNF- $\alpha$  concentrations were slightly lower at 24 h in patients in both arms of the study. The median TNF level in NAC-treated patients fell from 33 pg/ml at 0 h to 27 pg/ml at 24 h. Although the differences in TNF  $\alpha$

at 24 h were statistically significant ( $p=0.01$ , Wilcoxon signed rank test), the magnitude of the change was small ( $<0.5$  pg/ml/patient). TNF- $\alpha$  concentration changes between 0 h and 24 h in the placebo group were not significant ( $p=0.25$ , Wilcoxon signed rank test).

IL-10 concentrations fell significantly from 0 h to 24 h in both NAC- and placebo-treated individuals. Median IL-10 concentration in NAC recipients was 209 pg/ml (95%CI 94–953 pg/ml) prior to treatment and 186 pg/ml (95%CI 37–856 pg/ml) at 24 h ( $p=0.05$ , Wilcoxon signed rank test). Median IL-10 concentration prior to placebo was 566 pg/ml (95%CI 165–910 pg/ml) and after 24 h was 279 pg/ml (95%CI 47–851 pg/ml,  $p=0.02$ ).

There were no significant changes in IL-6 or interferon- $\gamma$  levels. The median IL-6 concentration in volunteers who received NAC was 20 pg/ml (95%CI 12–785 pg/ml) prior to treatment and 12 pg/ml (95%CI 10–454 pg/ml) 24 h later. Median IL-6 concentration prior to placebo was 81 pg/ml (95%CI 14–377 pg/ml) and at 24 h was 31 pg/ml (95%CI 11–929 pg/ml). Interferon- $\gamma$  concentrations varied little (Table 2).

## Discussion

This pilot study suggests the possibility that NAC accelerated recovery from severe malaria. Raised serum lactate levels returned to normal twice as quickly in patients who received NAC (median 21 h, 95%CI 12–36 h) compared with patients who received placebo (median 42 h, 95%CI 30–84 h;  $p=0.002$ , Figure 1). Raised blood lactate is of major prognostic importance in both adult and paediatric severe malaria.<sup>12,13</sup> NAC adjunctive therapy also tended to shorten the duration of time during which antimalarial drugs had to be given intravenously, but differences between NAC and placebo were not statistically significant.

Surprisingly however, the benefits conferred by NAC did not appear to be attributable to effects on TNF or other cytokines. NAC only reduced TNF concentration from 33 pg/ml to 26 pg/ml at 24 h, and had little effect on interferon- $\gamma$ , interleukin-6 and interleukin-8 (Table 2). Subtle cytokine changes could have been missed however, since the sample size was relatively small and cytokine receptor assays were not performed. High levels of parasitaemia, IL-6 and CPK, as well as low IL-10 levels, have been linked to severe malaria.<sup>1,15,16</sup> There was a non-significant trend for parasite count and IL-6 concentration to be higher in individuals randomized to the placebo arm, and for high CPK concentrations and low IL-10 levels to be more prominent in the NAC group (Table 1).

How then might NAC be acting? Cytoadherence of *Plasmodium falciparum*-infected red blood cells to post capillary venular endothelium is an important determinant of the pathogenesis of severe malaria complications.<sup>17</sup> Gruarin *et al.* showed after our study was completed that NAC inhibited cytoadherence to CD36-expressing cells and dissolved preformed aggregates. Unfortunately we did not examine peripheral blood films after NAC administration for the presence of more mature parasites. Gruarin *et al.* suggested that their findings might offer a new therapeutic approach for severe malaria.<sup>18</sup> We agree. NAC is an unusually safe drug<sup>9</sup> and our preliminary findings suggest that it could be of benefit to patients hospitalized with malaria (Figure 1). However, the most important measure of treatment outcome in severe malaria is survival. Because there is a very low risk of causing harm by administering NAC and possible benefit, larger trials of NAC adjunctive therapy in severe *Plasmodium falciparum* malaria, with mortality as an endpoint, appear to be warranted.

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