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Effect of zinc on the treatment of Plasmodium falciparum malaria in children: A randomized controlled trial

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Effect of zinc on the treatment of *Plasmodium falciparum* malaria in children: a randomized controlled trial[1–3]

The Zinc Against Plasmodium Study Group

ABSTRACT

Background: Zinc supplementation in young children has been associated with reductions in the incidence and severity of diarrheal diseases, acute respiratory infections, and malaria.

Objective: The objective was to evaluate the potential role of zinc as an adjunct in the treatment of acute, uncomplicated falciparum malaria; a multicenter, double-blind, randomized placebo-controlled clinical trial was undertaken.

Design: Children (n = 1087) aged 6 mo to 5 y were enrolled at sites in Ecuador, Ghana, Tanzania, Uganda, and Zambia. Children with fever and ≥ 2000 asexual forms of *Plasmodium falciparum*/J.L in a thick blood smear received chloroquine and were randomly assigned to receive zinc (20 mg/d for infants, 40 mg/d for older children) or placebo for 4 d.

Results: There was no effect of zinc on the median time to reduction of fever (zinc group: 24.2 h; placebo group: 24.0 h; P = 0.37), a ≥75% reduction in parasitemia from baseline in the first 72 h in 73.4% of the zinc group and in 77.6% of the placebo group (P = 0.11), and no significant change in hemoglobin concentration during the 3-d period of hospitalization and the 4 wk of follow-up. Mean plasma zinc concentrations were low in all children at baseline (zinc group: 8.54 ± 3.93 J.mol/L; placebo group: 8.34 ± 3.25 J.mol/L), but children who received zinc supplementation had higher plasma zinc concentrations at 72 h than did those who received placebo (10.95 ± 3.63 compared with 10.16 ± 3.25 J.mol/L, P < 0.001).

Conclusion: Zinc does not appear to provide a beneficial effect in the treatment of acute, uncomplicated falciparum malaria in preschool children. *Am J Clin Nutr* 2002;76:805–12.

KEY WORDS Zinc, malaria, *Plasmodium falciparum*, preschool children, Africa, Ecuador

INTRODUCTION

Childhood malaria is a major public health problem worldwide, with an estimated 2 million children dying of malaria yearly, primarily because of *Plasmodium falciparum* and its complications (1). Approximately 400 million people are estimated to suffer from malaria morbidity annually; two-thirds of those people reside in sub-Saharan Africa.

Prompt diagnosis and early treatment continue to be the mainstays of the current approach to malaria control (2). Second-line drugs are often required to deal with chloroquine resistance, but their high cost and greater potential for adverse effects restrict their use in malaria-endemic countries. Because of the increasing prevalence of malaria, its associated morbidity and mortality in children, and the progressive increase in the resistance of the parasite to antimalarial drugs (3), new treatment options are desperately needed.

Children < 5 y old in malaria-endemic areas are at risk of protein-energy malnutrition, as well as deficiencies in micronutrients including zinc (4). Zinc deficiency in humans leads to growth retardation, thymic atrophy, lymphopenia, impaired T and B lymphocyte function, impaired chemotactic activity of neutrophils, and a reduction in thymulin activity, interferon-')' concentrations, and the number of CD4 (helper) lymphocytes (5). These alterations in the cellular and humoral functions may increase host susceptibility to *P. falciparum* (5, 6). Zinc supplementation of children in developing countries has resulted in improvement of delayed cutaneous hypersensitivity (7) and an increase in CD4 lymphocytes (8). Zinc supplementation has been shown to reduce the incidence of diarrhea and pneumonia (9) and to be beneficial when used as adjunctive therapy for acute diarrhea (10, 11).

A community-based zinc supplementation trial in The Gambia designed to evaluate the effect of zinc on growth found a trend toward fewer health center visits for malaria by children who received zinc (12). In Papua New Guinea, zinc supplementation in preschool children reduced malaria-attributable health center visits by 38% (13). On the basis of the therapeutic benefits of zinc for acute and persistent diarrhea, zinc's crucial role in immune system function, and recent evidence that zinc supplementation appears to reduce malaria morbidity, we examined the hypothesis that zinc given as an adjuvant to standard antimalarial therapy would improve the outcomes of acute episodes of *P. falciparum* malaria in children.

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[2] Supported by a Cooperative Agreement between Harvard University and the Office of Health and Nutrition of the US Agency for International Development and by Hermes Arzneimittel GmbH (Munich, Germany), which also supplied the zinc and placebo used in the study.

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SUBJECTS AND METHODS

Study design and sites

The study was a multicenter, double-blind, randomized placebo-controlled clinical trial at the following sites: Hospital Delfina Torres (Esmeraldas, Ecuador), Komfo Anokye Teaching Hospital (Kumasi, Ghana), Kisarawe District Hospital (Kisarawe, Tanzania), Mpigi Health Center (Mpigi, Uganda), and Arthur Davison Children's Hospital (Ndola, Zambia). These sites serve a mixture of urban and rural populations in Africa and Latin America. The 4 African sites are in malaria-hyperholoendemic zones, whereas Ecuador is in a hypoendemic zone. Subject enrollment took place between December 1998 and May 2000.

Study population

All children aged 6–60 mo who presented to the participating health institution for evaluation of fever were screened for the study. A finger-stick blood sample was taken from children with an axillary temperature ≥ 37.5 0C. Children with ≥ 2000 asexual forms of *P. falciparum*/J.L in a thick blood smear were considered eligible for enrollment into the trial. If the child did not meet any of the exclusion criteria and if his or her parent or caretaker was willing to give written, informed consent, then the child was enrolled in the study. Exclusion criteria included a hemoglobin concentration < 70 g/dL; severe malaria as defined by the presence of any of the following: cerebral malaria, severe anemia, renal failure, pulmonary edema, hypoglycemia, shock, spontaneous bleeding, or repeated convulsions (14); non-*P. falciparum* or mixed *Plasmodium* infections; concurrent severe infections (ie, lower respiratory infection, acute otitis media, pyelonephritis, typhoid fever, bloody diarrhea, meningitis, or measles); severe dehydration; malnutrition as defined by the Wellcome criteria (15) (ie, marasmus, kwashiorkor, or marasmic kwashiorkor); inability to tolerate oral medications or fluids; chronic illness (including tuberculosis, acquired immunodeficiency syndrome, severe congenital anomalies, sickle cell disease); and prior participation in this trial.

Randomization and blinding

Zinc and placebo tablets that were identical in appearance were packaged in identical polypropylene tubes labeled with subject identification numbers. Randomization was performed in blocks of 20 by using a table of random numbers and was stratified by site. Once a child was enrolled, the next container in the sequence was opened, and the corresponding regimen was provided to the child. Investigators, clinical staff, and patients were blinded to study group assignment. The study code was broken after completion of enrollment and follow-up of all study subjects.

Treatment specification

The zinc preparation that was used consisted of an effervescent, citrus-flavored tablet containing 25 mg Zn in the form of zinc sulfate (Biolectra Zink; Hermes Arzneimittel GmbH, Munich, Germany) dissolved in 25 mL water. The placebo, a zinc-free tablet having color, taste, and appearance similar to those of zinc sulfate and that was especially prepared by Hermes Arzneimittel GmbH, was put into solution in an identical fashion. The strong lemon-lime flavor of the solution effectively concealed the metallic taste of zinc, thus preventing study participants or personnel from being able to determine whether a preparation contained zinc or placebo.

Zinc was given in a total daily dose of 20 mg for children aged < 12 mo and 40 mg for children aged 12–60 mo. The zinc or placebo was administered in 2 equally divided daily doses during the first 3 d of the study and as a single dose on the fourth day. The drug was given under direct supervision 15–30 min before a meal. If the child vomited ≤ 15 min after drug administration, the dose was repeated.

Clinical care of subjects

Baseline evaluation

Demographic data, information on the use of antivector measures to prevent malaria or on recent antimalarial use, and significant medical history were obtained. A complete physical examination, including vital signs, anthropometric measurements, hydration status, neurologic status according to the Blantyre coma scale (16), and abdominal examination to measure liver and spleen size, was performed. Subjects were admitted to the inpatient ward for 48–72 h.

Inpatient care

Clinical monitoring of patients included measurement of axillary temperature every 4 h and physician evaluation every 24 h or more frequently if clinically indicated. In addition, standard nursing care was provided. All subjects received standard medical care for any concurrent illnesses that were present at baseline or that developed during the study. For these infections, the use of the antibiotics trimethoprim, sulfamethoxazole, erythromycin, and doxycycline was avoided because of their antimalarial effects. Paracetamol was administered for temperatures ≥ 38.5 0C at a dose of 10–15 mg/kg every 6 h.

Antimalarial therapy

Chloroquine was given as the first-line drug in accordance with the national treatment guidelines for malaria in all 5 countries. The following schedule was used: 10 mg/kg on day 0, 10 mg/kg on day 1, and 5 mg/kg on day 2. Tablets containing 150 mg base were used. If either a treatment or parasitologic failure occurred, subjects were changed to a standard dose of either amodiaquine or sulfadoxine as second-line antimalarial therapy. Treatment failure was defined as the presence of axillary temperature ≥ 37.5 0C and parasitemia of > 25% of the baseline value at 72 h. Parasitologic failure was defined as parasitemia of > 25% of the baseline value Swith resolution of fever (ie, temperature < 37.5 0C at 72 h). Children who developed cerebral malaria or who failed to respond to second-line therapy were treated with quinine. If, during the course of treatment, the condition of the patient deteriorated or complicated malaria developed, then the clinician had the option to change treatment as clinically indicated. Children with severe symptomatic anemia were given a transfusion of packed red blood cells. Discharge from hospital occurred once the fever had resolved (axillary temperature < 37.50C for ≥ 12 h) and there was a ≥ 75% reduction in parasitemia relative to baseline.

Outpatient follow-up

Parents were asked to bring the subject back to the study site at 72 h (if discharged at 48 h) and on the mornings of days 7, 14, and 28. Parents were instructed to bring the child to the study site immediately if he or she developed fever or other signs of illness at any time between discharge and day 28. If the patient did not

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return for follow-up, a research assistant went to the subject's home to locate him or her. At each follow-up visit, subjects were asked about any illnesses since the last visit, especially those manifested by fever, and about any use of an antimalarial drug. Blood smears were obtained and hemoglobin concentrations were measured at each follow-up visit.

Criteria for withdrawal from the study

If a subject, during the inpatient phase of the trial, developed coma or the inability to tolerate oral fluids or medicines, he or she was withdrawn from the assigned treatment arm of the study, but outcome data were still collected, including outpatient follow-up. If informed consent was withdrawn at any time during the trial, the subject was discharged from the study.

Laboratory evaluation

Giemsa-stained thick blood smears were made on admission (time 0); at 24, 36, 48, and 72 h; and on days 7, 14, and 28. The total parasite count per J.L was quantitated (17). A thick smear was declared negative after the viewing of high-power fields containing 500 white blood cells. Hemoglobin concentrations were determined by Hemacue (Angelholm, Sweden) on admission and on days 7, 14, and 28. Blood for plasma zinc was taken on day 0 before the administration of the study drug and then at 72 h. Samples were obtained just before meals. Venous blood was drawn with zinc-free syringes and placed into heparinized zinc-free tubes. Blood was immediately centrifuged and plasma was transferred into zinc-free tubes with a plastic zinc-free pipet and frozen at −20 0C. Plasma zinc was assayed by atomic absorption spectrophotometry at the University of Colorado Center for Human Nutrition.

Outcomes

Primary outcomes were the number of hours to the resolution of fever (fever resolution time was defined as the time at which axillary temperature remained < 37.5 0C for 12 consecutive hours) and the proportion of subjects with ≥ 75% reduction in parasitemia at 72 h (compared with parasitemia values on admission). Secondary outcomes were the proportion of subjects who were aparasitemic at 72 h and on days 7, 14, and 28; the mean change in hemoglobin from day 0 to days 7, 14, and 28; and the change in mean plasma zinc concentrations between time 0 and 72 h.

Quality control

Clinical measurements

All investigators took part in a protocol development workshop during which the protocol was designed and a consensus was reached on the details of clinical care of children with acute malaria. Uniformity in the application of inclusion and exclusion criteria, as well as in case management, was emphasized, and a study manual was written. Training of study personnel and enrollment of 5 pilot subjects were performed at all sites. Technical staff made site visits at the beginning of enrollment to assess study procedures.

Laboratory measurements

Hemoglobin measurements were made at all sites by using the same portable machine (Hemacue) and technique. Blood smears were performed in an identical manner at all sites. Internal quality control consisted of the duplicate reading of a 10% subsample of smears by an experienced parasitologist. External quality control on a 5% sample of slides from all sites was done by an experienced parasitologist who was not otherwise involved in the study (18). The comparison of the results of the external quality control of blood smears performed by an independent reader with the blood smear results found by the study parasitologist at each site yielded a mean (± SD) difference of 0.14 ± 3.55 between the 2 groups in log-transformed parasite density counts. This means that there was an average difference in parasite density counts of 15% (with a large amount of variation) between the blood smear readings done at each site and those done by the external quality-control parasitologist. However, this difference was not significant ($P = 0.59$).

Data management, statistical power, and statistical methods

All case report forms were checked for missing, discrepant, and illogical responses by the study supervisor. The Applied Research for Child Health Project (Cambridge, MA) was the trial's data coordinating center. Double data entry was performed; one entry was done at the site, and the second entry and validation were done at the Applied Research for Child Health Project. Data entry and management were carried out with EPI-INFO software, version 6.04c (Centers for Disease Control and Prevention, Atlanta) and Integrated Microcomputer Processing System, version 3.1 (US Bureau of the Census, Washington, DC).

To obtain the proper sample size for the trial, we calculated the statistical power necessary to examine the effect of the supplements on each of the 2 primary outcomes, and the larger of the 2 sample sizes thus obtained was used. It was estimated that 25% of the children in the placebo group would fail to achieve ≥ 75% reduction in parasitemia at 72 h. Assuming a potential efficacy of 30% for the zinc regimen and a 10% loss to follow-up, 1025 subjects were required for enrollment to answer the question with a power of 80% and a two-tailed level of significance of 5%. The assumption of a 30% beneficial effect of zinc supplements was based on a study from Papua New Guinea, in which zinc supplementation was associated with a 30% reduction in health center visits for malaria (19).

All analyses were made according to the intention-to-treat principle. Frequencies of outcomes between treatment and control groups were compared by using the chi-square test (20). Differences in medians between treatment and control groups were compared by using the Wilcoxon rank-sum test (20). The analysis of repeated-measures data used mixed-models regression (21). Differences in the hours to reduction in fever were compared by using the log-rank test for homogeneity for Kaplan-Meier survival curves (22). In a subgroup analysis for the detection of effect modifiers, we used binomial regression with the log link function and simultaneously controlled for the study site (23). We used SAS software, version 7.0 (SAS Institute Inc, Cary, NC) for statistical analysis.

Ethical approval of the study was obtained from the institutional review boards at each site and at the Harvard School of Public Health. Written, informed consent was obtained from the parent or guardian of each subject. A data safety monitoring board consisting of 2 experts in infectious diseases and a biostatistician not otherwise involved in the trial performed a review of serious adverse events midway through the study.

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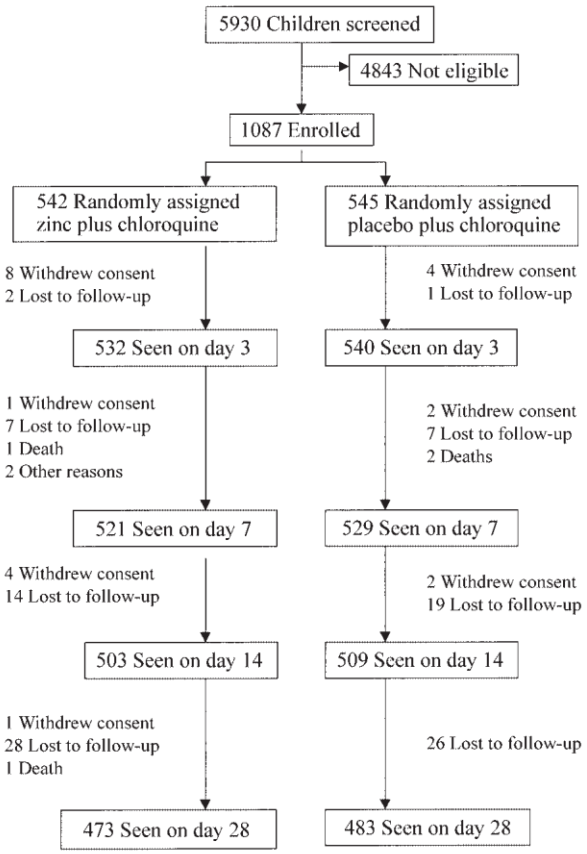

FIGURE 1. Study profile.

RESULTS

A total of 5930 persons were screened at the 5 study sites, and 1087 subjects were enrolled (**Figure 1**). The distribution of enrollees was 77 children in Ecuador, 214 in Ghana, 260 in Tanzania, 276 in Uganda, and 260 in Zambia. The most common reasons for ineligibility included the absence of fever at the time of health center presentation and the presence of severe anemia, malnutrition, or infection with *Plasmodium vivax*. The ratio of screened to enrolled patients was not equal at the sites. The African sites screened =2 patients for each patient enrolled, whereas the site in Ecuador screened an average of =35 patients for each patient enrolled. This imbalance in the rate of ineligibility stemmed primarily from the need to exclude patients who did not have malaria as a cause of their fever; moreover, of those in Ecuador who did have malaria, about one-third had *P. vivax*, but this *Plasmodium* species was rarely encountered in the African sites. The baseline characteristics of the treatment (zinc) and control (placebo) groups were not significantly different (**Table 1**).

Zinc or placebo treatment

Overall, 98.3% of the planned doses of zinc or placebo were given to the children. The percentage of children who did not take the planned dose did not differ significantly between the zinc and placebo groups. The amount of vomiting during the course of hospitalization did not differ significantly between the 2 groups (12.7% in the zinc group and 12.1% in the placebo group, $P = 0.76$).

TABLE 1
Baseline characteristics of subjects[1]

	Zinc group (n = 542)	Placebo group (n = 545)
Population characteristics		
Age (mo)	26.4 ± 15.9[2]	27.0 ± 15.9
Mother's school attendance (y)	5.7 ± 3.6	5.7 ± 3.6
Male (%)	52	52
Breast-feeding (%)	41	39
Use of bed nets (%)	22	24
Antimalarial use in previous 7 d (%)	37	33
Nutritional status		
Weight-for-age (z score)	−1.08 ± 1.1	−1.16 ± 1.2
Height-for-age (z score)	−1.43 ± 1.5	−1.53 ± 1.5
Weight-for-height (z score)	−0.16 ± 1.2	−0.21 ± 1.3
Midupper arm circumference (cm)	15.0 ± 1.4	14.9 ± 1.4
Clinical data		
Axillary temperature (°C)	38.7 ± 0.9	38.6 ± 0.9
Splenomegaly (%)	4	3
Laboratory data		
Parasitemia (/J.L)[3]	19 283 (1 500–435 920)	17 448 (225–430 000)
Hemoglobin (g/L)	92 ± 16	93 ± 16
Plasma zinc		
(J.mol/L)	8.5 ± 3.9	8.3 ± 3.3
(J.g/dL)	55.9 ± 25.7	54.5 ± 21.6

[1] There were no significant differences between the groups.
[2] $\bar{x}$ ± SD.
[3] Geometric $\bar{x}$; range in parentheses.

Severe adverse events occurred in 35 subjects (**Table 2**). These included 2 episodes of cerebral malaria, 26 episodes of severe anemia requiring transfusion, 3 episodes of febrile convulsions, and 4 deaths. The incidence of severe adverse events did not differ significantly between the 2 groups. The data safety monitoring board reviewed the severe adverse events and concluded that these were unlikely to be related to the study intervention.

Primary and secondary outcomes

Because there was no significant interaction between the effect of the supplements (zinc or placebo) and the sites (African sites or Ecuador) on the primary outcomes, all analyses include the data pooled from the 5 participating study sites. The Kaplan-Meier survival curves for time to resolution of fever are shown in **Figure 2**. The median time to reduction of fever did not differ significantly between the groups (24.2 compared with 24.0 h, $P = 0.37$). Of the 965 subjects whose fever resolved during the first 72 h, 638 received paracetamol, whereas 327 did not. There was no significant difference between the zinc and placebo groups

TABLE 2
Summary of severe adverse events[1]

Adverse event	Zinc group (n = 542)	Placebo group (n = 545)
Cerebral malaria (n)	1	1
Severe anemia (n)	17	9
Febrile convulsion (n)	1	2
Death (n)	2	2
Total (n)	21	14

[1] There were no significant differences between the groups.

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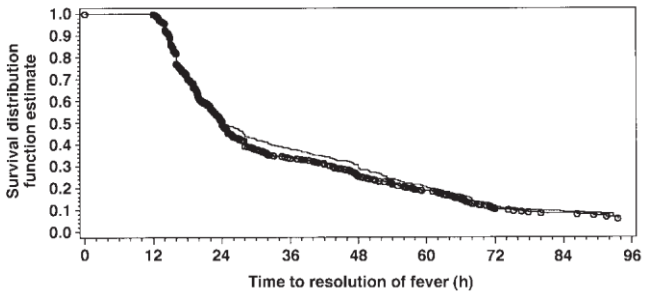

FIGURE 2. Time to resolution of fever in the zinc (—) and placebo (—O—) groups. The median time to resolution of fever was not significantly different between the groups.

in the number of children who received paracetamol. Of the children receiving paracetamol, 259 did not receive a dose before the resolution of fever, whereas 379 were given paracetamol at some point before the resolution of fever. Because fever resolution was defined as axillary temperature < 37.5 0C for 12 consecutive hours, it was possible for study subjects to meet the definition of fever resolution and then have a recurrence of fever. Of the 259 subjects who received their first dose of paracetamol after fever resolution, 258 had a temperature ≥ 37.5 0C after fever resolution. However, in 97% (368 of 379) of the children, the fever did not resolve for at least 13 h after they were last given paracetamol. Thus, paracetamol use was unlikely to have had any effect on the duration of fever.

The proportion of children whose parasitemia value was reduced ≥ 75% in the first 72 h in the zinc group was 73.4% (398 of 542) and that in the placebo group was 77.6% (423 of 545) (χ^2 = 2.57, P = 0.11). There also was no significant difference between the groups in the percentage of children who required second-line antimalarial therapy during the period of hospitalization (zinc group: 12.6%; placebo group: 12.3%).

There were no significant differences in secondary outcomes between the 2 groups, except for the change in plasma zinc concentration between 0 and 72 h. An analysis of data from the African sites alone (ie, excluding the data from Ecuador) revealed no significant difference between the zinc and placebo groups in any of the secondary outcomes. With antimalarial treatment, about one-fifth of the children in both groups were aparasitemic at 72 h

TABLE 3
Percentage of aparasitemic children and mean change in hemoglobin concentration in the zinc and placebo groups[1]

Variables	Zinc group	Placebo group
Aparasitemic subjects (%)		
72 h	20.2 [100 of 496]	21.5 [108 of 502]
7 d	50.9 [258 of 507]	54.7 [280 of 512]
14 d	54.6 [265 of 485]	53.6 [261 of 487]
28 d	59.9 [277 of 462]	55.6 [263 of 473]
Change in hemoglobin from day 0 (g/L)		
7 d	−4.9 ± 17.2 [502]	−4.8 ± 18.4 [519]
14 d	3.4 ± 17.9 [476]	2.6 ± 17.4 [477]
28 d	9.3 ± 18.8 [454]	7.5 ± 18.9 [463]

[1] $\bar{x}$ ± SD; *n* in brackets. There were no significant differences between the groups.

TABLE 4
Plasma zinc concentrations at baseline and 72 h[1]

	Plasma zinc concentration	
Study group	Baseline	72 h
Zinc group		
(J.mol/L)	8.54 ± 3.93	10.95 ± 3.63[2]
(J.g/dL)	55.9 ± 25.7	71.6 ± 23.7[2]
Placebo group		
(J.mol/L)	8.34 ± 3.25	10.16 ± 3.25[2]
(J.g/dL)	54.5 ± 21.3	66.5 ± 21.3[2]

[1] $\bar{x}$ ± SD. The data were analyzed with repeated measures with the use of mixed models with an interaction for treatment and time. There was a significant interaction between group and time, P = 0.038.
[2] Significantly different from baseline, P < 0.001.

(**Table 3**). The proportion of children in both groups who were aparasitemic had increased to nearly 60% by day 28. More than 19% (207 of 1087) of the children received second-line antimalarials between days 7 and 28.

At 72 h, plasma zinc concentrations had increased in both groups (**Table 4**). Although there was no significant difference in baseline plasma zinc concentrations between the groups (Table 1), the children who received zinc supplements had significantly larger increases in zinc concentrations between baseline and 72 h than did the children who received placebo [0.59 ± 0.28 J.mol/L (3.8 ± 1.8 J.g/dL), P = 0.038].

The primary outcome of the reduction of parasitemia by 75% at 72 h was modeled by using binomial regression (22). No significant differences in outcomes between the groups were found after controlling for age group, weight-for-age *z* score, maternal education, breast-feeding, use of bed nets, prior chloroquine use, second-line antimalarial use, baseline plasma zinc concentration, and study location. The presence of effect modifiers was tested by using binomial regression in which study location was controlled for. No difference in treatment effect was found with any of the categories of effect modifiers that were tested (sex, baseline plasma zinc, baseline hemoglobin, weight-for-age *z* score, height-for-age *z* score, weight-for-height *z* score, and baseline parasitemia).

DISCUSSION

We found that zinc as an adjuvant to the treatment of uncomplicated *P. falciparum* malaria in children aged 6–60 mo had no effect on the duration of fever or on the reduction of parasitemia at 72 h. In addition, there was no apparent effect of the intervention on hematologic or parasitologic measures during the 4 wk of follow-up.

The 2 previous studies that showed a beneficial effect of zinc in malaria were community-based prevention trials (12, 13). The first, a placebo-controlled study in which preschool children in rural Gambia received zinc supplements twice weekly for 1.25 y (12), showed a trend toward a reduction in the number of clinic visits for malaria (P = 0.09). The small sample size of that study most likely did not provide adequate power to allow the detection of an effect of zinc supplementation on the rates of clinic visits for malaria. In addition, the twice-weekly dosing interval may have been responsible for the failure of the zinc supplement to significantly increase zinc concentrations in plasma or hair

relative to those found in the placebo group. In a more recent, larger supplementation trial in preschool children in Papua New Guinea (13) in which zinc was administered daily for 46 wk, there was a significant reduction in episodes of *P. falciparum* malaria. The greatest effect of the zinc intervention was observed in children with *P. falciparum* episodes accompanied by parasitemia ≥ 100 000 parasites/J.L. However, in contrast to these 2 studies, a recent study in Burkina Faso found that 6 mo of zinc supplementation had no effect on the incidence of symptomatic falciparum malaria, severity of malaria episodes, or other malariometric indexes (24).

The lack of efficacy of zinc in our trial may be due to a number of factors. First, the duration of the supplementation in this study was purposefully short. The intervention was designed to be administered in conjunction with antimalarial therapy in a way that would be practical in the developing world. Previous studies examining the therapeutic role of zinc in acute infections have also taken this approach (10, 11, 25). The beneficial role of zinc in the treatment of diarrhea may be due to a direct effect of zinc on intestinal function by virtue of improved absorption of water and electrolytes (26, 27), early regeneration of the epithelial lining (28, 29), and improved production of brush border digestive enzymes (30, 31). In contrast, for zinc to have a beneficial effect on malaria, there may need to be improvements in the functioning of the immune system. It is not known how long it takes zinc supplementation to lead to a resolution of abnormalities of immune function when it is used to treat zinc deficiency. Three days of zinc supplementation may have been insufficient to allow for the improvement of immune function in children with underlying zinc deficiency, whereas longer periods of supplementation clearly do lead to better immune function (5, 7, 8). The duration of zinc supplementation may explain the difference between our results and those of the studies of zinc that found this micronutrient to be beneficial in the prevention of malaria (12, 13).

Second, treatment with zinc would be unlikely to be beneficial if the study subjects had normal zinc nutritional status. The study participants, however, resided in areas where there is a high risk of zinc deficiency because of cereal-based diets, which are relatively low in zinc and high in phytates (32, 33). Accurate assessment of zinc status is difficult and optimally should involve the use of multiple measures (34). However, many of the more sophisticated measures of zinc status such as platelet, lymphocyte, or tissue zinc concentrations are not practical for large field studies in developing countries. In addition, these measures have not been validated as markers of zinc status. Although plasma zinc concentrations are commonly used as a surrogate marker of zinc status in many clinical trials, plasma zinc represents < 0.2% of total body zinc stores, and there is a poor correlation between plasma zinc concentrations and body stores (35). In addition, it is recognized that acute infections and febrile illnesses decrease plasma zinc concentrations, although the extent and timing of this decrease are unclear (36). The magnitude of the decrease in plasma zinc concentrations in persons with acute infections has been shown in animal models to be a function of the stage, duration, and severity of the infection (37–39). Although some community-based studies found minimal to no suppression of plasma zinc concentrations in infected children compared with that in uninfected children (40–42), it is possible that the severity of illness in these studies was not great enough to cause a significant decrease in plasma zinc. The baseline plasma zinc concentrations in our study cohort were initially very low, but they increased as the acute episode of malaria resolved. This suggests that the plasma zinc concentration had been depressed as part of the acute phase response. However, even after resolution of fever, the plasma zinc concentrations remained relatively low, with a mean concentration of < 10 J.mol/L at 72 h in the placebo group. This suggests that, in our study population, there may have been a combination of low plasma zinc concentration in response to the acute infection and an underlying zinc deficiency. Because the children who were treated with zinc had significantly higher plasma concentrations at 72 h, it appears that there was good bioavailability of the supplement. However, the absolute magnitude of the differences in plasma zinc between the zinc and placebo groups was relatively small (0.7 J.mol/L, or 5.1 J.g/dL). The physiologic or clinical significance of this relatively small difference is unclear.

Third, the dose of zinc used may not have been adequate. Higher zinc doses may conceivably have had a stronger effect, but we felt that a dose that was 4 times the US recommended dietary allowance (43) was a reasonable one. The mean daily dose, based on the mean baseline weights of the children who received zinc, was 2.5 mg/kg for children aged 6–11 mo and 3.4 mg/kg for those aged 12–60 mo. This amount of zinc was well tolerated, resulted in a significantly higher final plasma zinc concentration than did placebo, and was not associated with any serious adverse events in our study. High-dose zinc (6.0 $mg \cdot kg^{-1} \cdot d^{-1}$) has been associated with increased mortality in severely malnourished children (44). In addition, high doses of zinc have been associated with the impairment of immune responses in healthy adults (45).

Fourth, the use of paracetamol may have masked a potential effect of zinc on parasitemia. A randomized trial in Gabon of children who were being treated for *P. falciparum* malaria found that a significantly greater amount of time was needed for parasite clearance in children treated with paracetamol than in those treated with mechanical antipyresis alone (46). Although the results of that study suggest that the approach used to manage fever in children with malaria may have an influence on the duration of parasitemia, it is unlikely that the use of paracetamol in our study interfered with a potential effect of zinc, because the proportion of children treated with paracetamol in the placebo and zinc groups was similar, and fever was not resolved in most of the children for > 12 h after receipt of paracetamol.

Unlike the situation at the site in Ecuador, where many children were excluded from participation because of the absence of malaria or the presence of *P. vivax*, the ineligibility rate for the African sites was < 50%. The aim of this study was to evaluate the intervention in a population that would be representative of those children presenting to urban or rural health care centers with acute, uncomplicated falciparum malaria. The final cohort of children fit this description. Because of the decision to focus only on malaria due to *P. falciparum* (which is the major source of morbidity and mortality worldwide), the results of this study cannot be generalized to areas where other species of *Plasmodium* are commonly encountered. However, the findings of this study can be applied to many developing countries where *P. falciparum* is endemic, because the study's multicenter design with site-by-site differences in the extent of underlying immunity to malaria and in the intensity of malaria transmission allows a high degree of generalizability. Moreover, the large sample size reduced the chance of a type 2 error. Multiple measures of quality control, adequate blinding and randomization, and excellent intrasite agreement in reading blood smears all ensured the internal validity of the trial.

In conclusion, in this large randomized trial, we found no evidence that supplemental zinc, given as an adjuvant to standard chemotherapy, was helpful in the treatment of acute malaria in young children. However, because there may be benefits of zinc supplementation in the prevention of malaria morbidity (12, 13), additional community-based studies are needed to clarify the prophylactic role of this essential micronutrient in countries with endemic malaria.

The members of the Zinc Against Plasmodium Study Group are listed below by country.

Ecuador: Fernando Sempértegui directed and oversaw implementation of the study, and Bertha Estrella oversaw implementation. Franklin R Toapanta and Darwin S Torres were the study clinicians, and Dheyanira E Calahorrano was responsible for laboratory operations.

Ghana: Kojo Yeboah-Antwi and Emmanuel Addo-Yobo coordinated and oversaw implementation. Paul Arthur (deceased) was the site's technical consultant, and Sam Newton was the study clinician.

Tanzania: Zul Premji oversaw implementation. Mloka Hubert was the study clinician, and Cyprian S Makwaya coordinated the site's data entry and statistical analyses.

Uganda: Freddie Ssengooba coordinated implementation, and Joseph Konde-Lule oversaw implementation. Emmanuel Mukisa was the study clinician.

United States: Davidson H Hamer coordinated the entire study, organized the protocol development and data analysis workshops, and carried out site visits; William MacLeod supervised the data coordination center, organized the data analysis workshop, and oversaw the statistical analysis of the combined site results; and Christopher Duggan assisted with the organization of the protocol development and data analysis workshops and provided technical assistance throughout the study. Wafaie Fawzi assisted with the organization of the protocol development workshop, carried out site visits, and provided technical assistance throughout the study; Jonathon Simon assisted with the organization of the protocol development workshop and provided technical assistance throughout the study.

Zambia: Victor Mwanakasale oversaw implementation of the study. Modest Mulenga assisted with the initiation of the trial, Thomas Sukwa was the site consultant, and John Tshiula was the study clinician.

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