

Randomized, Controlled Trial of Single Versus 3-Times-Daily Ferrous Sulfate Drops for Treatment of Anemia

Stanley Zlotkin, MD, PhD*†; Paul Arthur, MD, MPH§||; Kojo Yeboah Antwi, MDS; and George Yeung, PhD‡

ABSTRACT. *Objective.* Adherence to treatment of iron-deficiency anemia often is poor in both developed and developing countries. The current standard therapy is ferrous sulfate drops (or syrup) administered 3 times daily. It is possible that adherence would improve with a single-dose daily treatment regimen. We compared the use of single versus 3-times-daily ferrous sulfate drops, at the same total iron dose, on treatment of anemia in infants.

Methods. To obtain a large enough cohort of anemic subjects, we performed the study in rural Ghana. Using a prospective, randomized, controlled design, we studied 557 anemic children (age range: 6–24 months; hemoglobin values: 70–99 g/L). One group ($n = 280$) received ferrous sulfate drops once daily (40 mg elemental iron), and the control group ($n = 277$) received ferrous sulfate drops 3 times per day (total dose, 40 mg elemental iron). Treatment lasted for 2 months. Hemoglobin and serum ferritin values were measured at baseline and at the end of the study.

Results. Successful treatment of anemia (hemoglobin >100 g/L) occurred in 61% of the single-dose and in 56% of the 3-times-daily group. Geometric mean ferritin levels increased significantly in each group from baseline to the final visit. Side effects were minimal and similar between the 2 groups.

Conclusion. A single versus a 3-times-daily dose of ferrous sulfate drops over 2 months resulted in a similar rate of successful treatment of anemia, without side effects. To our knowledge, this is the first demonstration of the use of a single-dose daily regimen to treat anemia. Although not examined in the current study, use of a single-dose daily regimen may improve adherence to treatment of anemia in infants. *Pediatrics* 2001;108:613–616; iron, ferrous sulfate drops, anemia, randomized controlled trial, single versus 3-times-daily treatment.

ABBREVIATION. IDA, iron-deficiency anemia.

From the *Departments of Paediatrics and Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada; †Division of Gastroenterology and Nutrition and Programs in Metabolism and Integrative Biology, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada; §Kintampo Health Research Centre, Ministry of Health, Kintampo, Ghana; and ||London School of Hygiene & Tropical Medicine, Maternal Child Epidemiology Unit, London, United Kingdom.

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Reprint requests to (S.Z.) Division of GI/Nutrition, The Hospital for Sick Children, 555 University Ave, Toronto, ON M5G 1X8, Canada. E-mail: zlotkin@sickkids.on.ca

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Iron deficiency, the most common nutritional problem in the world, affects two thirds of children in most developing nations.¹ In Ghana, the recent national survey revealed an 83.5% rate of anemia in preschool children, whereas in the United States, the estimated prevalence is 3% to 5%.^{2,3} Iron-deficiency anemia (IDA) is a leading cause of morbidity and mortality worldwide. Numerous studies have demonstrated that moderate IDA (hemoglobin <100 g/L) is associated with depressed mental and motor functions that may not be reversible.^{4–6} Although many developing countries recommend iron supplementation for pregnant women and young children and treatment of documented anemia, adherence to treatment often is poor. It has been suggested that alternative treatment regimens and iron formulations may improve outcomes.^{7,8}

At least 4 important variables may influence the success of treatment of IDA with oral iron: the dose per 24 hours, the frequency at which the dose is provided, the form in which the dose is provided, and the patient's adherence to treatment. The issue of what is considered to be the appropriate dosage of iron has been well studied. The present criterion standard for treatment is 1.5 to 2.0 mg of elemental iron per kilogram of body weight provided 3 times per day (total dose: 4.5–6.0 mg/kg/d).⁹ The recommendation is to provide therapy for 2 months, then, to enhance iron stores, to continue therapy for an additional 1 month or more.

With regard to the issue of the frequency of administration, alternative therapeutic regimens, such as weekly or alternate-day dosing, have been suggested.^{10,11} However, too few studies have been completed in infants and young children to warrant deviation from the current criterion standard, especially for treatment (rather than prevention) of anemia.¹² Moreover, a single weekly oral treatment-dose of iron may be high enough to cause side effects that may preclude its routine use for infants. We believe that a single daily dose is not too high to cause side effects and would achieve better adherence compared with the standard 3-times-daily dose.

In the randomized, controlled trial that we report here, we tested the hypothesis that the response to treatment of anemia would be lower in patients after 2 months of once-daily treatment with ferrous sulfate drops compared with the same drops provided 3 times daily. Our objective, therefore, was to determine whether anemia would be treated successfully

by a single daily dose of iron or by the same dose given 3 times daily.

METHODS

Study Area, Participants, and Recruitment

The study took place between May and August 1999 in the field study area for the Kintampo Health Research Center, located in the Kintampo district of Ghana. This is a malaria-endemic area where the principle complementary food is a maize-based porridge. The prevalence of anemia in young children is estimated to be >80%, a significant proportion of which is attributable to iron deficiency.²

Eligible infants were identified from an existing surveillance database of births in the district. To be included in the study, infants had to be 6 to 18 months of age at the time of recruitment and have a hemoglobin concentration between 70 and 99 g/L, measured during a baseline assessment. Children who were severely anemic (hemoglobin <70 g/L) were excluded from the trial and treated.

Study Design

Because it would be unethical to provide a placebo to a child with anemia, we did not include a placebo control. After the baseline assessment, children were randomized individually to 1 of the 2 treatment groups. Randomization was done with sealed opaque envelopes that contained group designations, which were generated randomly by computer with Microsoft Access 97 (Microsoft Corporation, Seattle, WA). It was not feasible to blind the field staff or the mothers to the group to which the children were assigned. However, the people who were responsible for the laboratory and data analyses were blinded to the group designations.

The standard-dose group received ferrous sulfate drops (5 mg/kg/d of elemental iron, rounded to a total of 40 mg of elemental iron) provided in 3 equal doses per day. The intervention group received ferrous sulfate drops at the same dose (40 mg) provided daily in a single bolus. A single bottle of ferrous sulfate was dispensed to mothers in both groups once every 4 weeks.

During the baseline assessment, a written questionnaire was administered to collect demographic, nutritional, and health data for each infant. Field workers visited infants at 2-week intervals after the baseline visit, for a total of 5 visits. At each visit, a questionnaire about the side effects and adherence during the preceding 7 days was completed. Data collected about side effects included the incidence of diarrhea, constipation, and general discomfort after ingestion of the iron drops. Questions about adherence to treatment included whether the children objected to taking the iron and how many doses were missed. Field workers provided parents with oral educational reinforcement to maximize adherence to the intervention.

Capillary blood samples at baseline and final visits were obtained from a finger prick using aseptic techniques, and hemoglobin concentration was determined on the spot using a portable HEMOCUE B-hemoglobin photometer (Hemocue Inc, Angelholm, Sweden) by trained technicians using standardized techniques.¹³ Malaria parasite smears were taken (at the baseline visit only), and 500- μ L blood samples were collected and preserved in ice-lined cold boxes. Blood samples were returned to the base station within 6 hours of collection, where the serum was separated by centrifugation (10 minutes at 1300 RPM) before storage at -40°C. Serum ferritin was assayed in duplicate by a commercial enzyme-linked immunosorbent assay, using a Spectro Ferritin Kit (Ramco Laboratories, Houston, TX).¹⁴ Baseline and final ferritin samples from an individual patient were assayed on the same day (in a single batch) on one 96-well microtiter plate to minimize interassay variation. An external reference standard (Lyphochek Anemia Control; Bio-Rad, Anaheim, CA) was assayed in duplicate on each microtiter plate for the ferritin assay.

Sample Size and Power

The primary outcome was successful treatment of anemia (ie, the proportion of children with hemoglobin values \geq 100 g/L). On the assumption of 90% cure rates in the control group (drops 3 times/d) and 80% in the experimental group, with a type I error set at 0.05, and a 0.9 probability of detecting a true difference, the

final sample size estimate was 286 patients per group. We expected that 65% of the infants in the Kintampo region would have hemoglobin values within our target range (70–99 g/L), so a total of 880 infants were actually screened to obtain the needed number of patients for the study.

Data Processing and Analysis

Data forms were checked manually for completeness and consistency before submission for processing. Data were entered twice by 2 different data-entry clerks in Visual Fox Pro 6.0 (Microsoft Corporation), verified, and checked for range and consistency with customized data-entry and processing programs (Microsoft Access 97). Data queries were forwarded to the Kintampo field office and resolved, whenever possible, by rechecking original data forms or verified in a repeat home visit, if indicated. Monthly summary reports, including the entered data, were sent electronically from Kintampo to the Central Study Center in Toronto, Canada, by means of the Internet and an file transfer protocol host site.

Data were analyzed with Statistical Analysis Software, version 6.12 (SAS Institute, Inc, Cary, NC). The proportion of children who were treated successfully was compared between the groups with χ^2 analysis. Paired *t* tests were used to analyze the change in hemoglobin and ferritin measurements over time. Differences between groups in hemoglobin and ferritin measurements at the beginning and at the end of the study were assessed by analysis of variance (with proc General Linear Models in SAS 6.12). All analysis of ferritin values was conducted on log-transformed data because of their skewed frequency distribution. The acceptable level of statistical significance for all tests was *P* < .05.

Ethics Approval and Consent

Ethics approval for this study was obtained from the research ethics committees at The Hospital for Sick Children, the London School of Hygiene and Tropical Medicine, and Ghana's Ministry of Health through the Health Research Unit. Oral consent to conduct the study in the Kintampo district was obtained from the District Assembly of Elected Representatives. Consent to conduct the study in each village was obtained from village elders, and individual consent to participate in the study was obtained from the mothers of infants included in the study.

RESULTS

After the screening survey, 557 infants with hemoglobin concentrations between 70.0 and 99.9 g/L were randomized to treatment. Fifty-seven of the 557 infants (10.2%) were absent from the survey at the end of the trial. This loss was distributed similarly between the treatment groups; moreover, there was no difference in baseline characteristics between these infants and the rest who successfully completed the trial. Consequently, a total of 500 infants completed the second and final assessment, including blood sampling.

There were no significant differences in the mean age (12.80 ± 4.67 months for 3-times-daily group vs 12.98 ± 4.7 for the once-daily group; *P* = .75), hemoglobin (*P* = .23; Table 1), or ferritin (*P* = .87; Table 2) values between the 2 treatment groups at the start of the trial. Gender was represented equally between the groups (139 [51%] of 247 vs 135 [49%] of 274 boys in 3-times-daily vs once-daily drops).

At baseline, 318 (66.8%) of the 476 infants for whom blood samples were available tested positive for malaria parasites (160 [66.7%] of 240) in the 3-times-daily group versus 158 (66.9%) of 236. Eighteen samples were discarded for technical reasons (inadequate slide preparation), and malaria data were not obtained for 131 patients. There were no significant differences among the 2 groups in the

TABLE 1. Mean Hemoglobin and Percentage Anemic by Treatment Group at Baseline and After 2 Months of Treatment

	Once-Daily Drops (n = 247)	3-Times-Daily Drops (n = 253)
Baseline hemoglobin (g/L)*	88 (8)	87 (9)
Percentage anemic	100‡	100
Final hemoglobin†	102 (18)	100 (17)
Percentage anemic	38.7	43.7

Values are means ± standard deviation.

* Values at baseline ($P = .47$) and final visit ($P = .25$) were similar in both groups.

† Mean hemoglobin values significantly increased from baseline to the final visit in both groups ($P < .001$).

‡ Values are percentage anemic, where anemia is defined as hemoglobin values <100 g/L.

TABLE 2. Geometric Mean Ferritin Values and Range, by Treatment Group, at Baseline and After 2 Months of Treatment

	Once-Daily Drops (n = 217)	3-Times-Daily Drops (n = 222)
Baseline ferritin ($\mu\text{g/L}$)*	34.8	40.0
	0.13–369.7‡	0.16–366.4
Final ferritin†	101.2	106.8
	4.05–398.1	6.58–391.8

Data are geometric means and range; analysis was done with log-transformed values because ferritin values are not distributed normally.

* Values at baseline ($P = .866$) and final ($P = .995$) were similar in both groups.

† Mean ferritin increased significantly from baseline to the final visit in both groups ($P < .001$).

‡ Normal values are 12 to 400 $\mu\text{g/L}$ (27).

prevalence of positive malaria smears ($P = .95$). The World Health Organization recommendation for the treatment of malaria was followed during the study. That is, if a patient were asymptomatic (ie, no fever) yet had a positive malaria smear, then he or she was not treated. None of the patients at baseline were febrile, thus none were treated.

Hemoglobin

In both groups, there was a significant increase in hemoglobin concentrations from baseline to the end of the study ($P < .001$; Table 1). The change in hemoglobin concentrations was similar between groups.

Fifty-nine percent (294 of 500) of infants advanced from an anemic to a nonanemic state (hemoglobin values ≥ 100 g/L). This rate was similar between groups: 61% (155 of 253 patients) for the once-daily ferrous sulfate group and 56% (139 of 247 patients) for the 3-times-daily group ($P = .51$). The relative risk of remaining anemic after 2 months of treatment was 0.92 times lower for the once-daily group (95% confidence interval: 0.79–1.06; $P = .26$) than that for the 3-times-daily group, but the difference was not significant.

Ferritin

The geometric mean ferritin values at baseline were similar in both groups and somewhat higher than was expected, likely reflecting the burden of concurrent infection (Table 2). There was a significant increase after 2 months of treatment ($P < .0001$). The variance

for ferritin values was wide at both baseline and the end of the study, as is usual with the wide interindividual and analytic variance associated with this measure, especially in a malaria-endemic region.^{15,16}

Adherence to Treatment and Side Effects

For children who took drops 3 times daily, 80% (462 of 578 responses from the 4 monitoring visits) received all of the prescribed doses compared with 81% (487 of 602) in the once-daily group. There was no difference in adherence to treatment between the children whose anemia did or did not resolve. Missing values were similar among groups. Seventy-four percent (933 of 1277) of mothers reported that their children objected to taking the drops in some way (children cried, made a funny face, and tried to keep their mouth shut when the drops were administered). Reported side effects were rare and mild and consisted mainly of diarrhea. There were no differences between the groups (diarrhea reported in 108 [18.7%] of 578 of those in the 3-times-daily group vs 109 [18.1%] of 602 in the once-daily group; $P = .80$).

DISCUSSION

Both treatment arms resulted in similar increases in hemoglobin and ferritin from the beginning to the end of the study and had similar success rates in the treatment of anemia. To our knowledge, this is the first formal demonstration of the use of a single versus multiple daily dose of iron for the treatment of anemia, although the concept has been discussed previously.^{17,18}

There are a number of possible explanations for why nearly 40% of infants remained anemic after 2 months of treatment. Sixty-five percent of the infants in the study tested positive for malaria, which has been shown to be a significant contributor to the cause of anemia in young infants in highly endemic areas.^{16,19} The mechanism that causes anemia with malaria is multifactorial, including enhanced destruction of infected red blood cells, removal of uninfected as well as infected red blood cells by the spleen, an immune-mediated hemolysis, and bone marrow unresponsiveness to erythropoietin.²⁰ Parasitic infections, common in West Africa, also may have contributed to continuing blood losses, although less so in infants who were younger than 30 months²¹; and, *Helicobacter pylori* infection, also common in children in West Africa, is associated with occult blood loss and refractory anemia.^{22,23} We did not check for the presence of sickle cell disease or α thalassemia trait, both of which are prevalent in Ghana. Thus, it is very likely that those who did not respond to iron drops were anemic for reasons other than iron deficiency. We were unable to define iron status accurately to confirm this because of the limited access to laboratory facilities in the region. Even if facilities had been available, Das et al¹⁶ demonstrated in a carefully controlled study that in a malaria-endemic area, markers of iron status are notoriously unreliable. If we assume that the majority who did not respond were not iron deficient, then the rate of successful treatment with either intervention would be significantly higher than 60%.

The definition of anemia in the current study was based on a hemoglobin concentration below which adverse functional outcomes have been observed.⁴⁻⁶ Lozoff et al⁵ demonstrated that at a hemoglobin above 100 g/L, infants seemed to be protected from the adverse mental and motor outcomes of anemia. Had we used the World Health Organization definition of anemia (hemoglobin \leq 110 g/L), the number of infants who advanced from an anemic to a non-anemic state would have been proportionately lower: 39% (99 of 253) for the once-daily group and 30% (75 of 247) for the 3-times-daily group ($P = .24$), but still similar between groups.²⁴ One could argue the merits of either hemoglobin standard. We used the lower hemoglobin standard believing that it was more realistic in Ghana, with its extremely high prevalence of anemia and limited resources, to aim for a potentially achievable target.

Our findings suggest important policy and program implications for the treatment of anemia, although the protocol was not designed to measure directly the adherence to treatment with the different dosing regimens (the protocol was not designed as an effectiveness study). For the past 150 years or more, oral ferrous sulfate has been the primary therapeutic (and preventive) source of iron for the treatment of IDA.²⁵ When a soluble form of iron (eg, ferrous sulfate) is ingested in the proper dose, this intervention is effective. However, adherence to long-term ingestion of oral iron drops (in an unsupervised setting) often is poor.⁷ In fact, there is very little evidence of the large-scale effectiveness of iron supplementation in young children. An effectiveness trial in Romania that included more than 2000 infants demonstrated only a limited reduction in anemia prevalence of 6- to 9-month-old infants. Low parental compliance with the administration of multiple daily doses of iron drops was implicated as one explanation of the results.²⁶

Although the use of drops, even once daily, still is complicated by a strong and unpleasant taste and the staining of teeth if the drops are not placed carefully at the back of the infant's mouth or the teeth are not immediately wiped, from a practical perspective, the option of using drops once daily may improve adherence to treatment and thus the success rate for the treatment of anemia.

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