Therapeutic Values of Different Routes of Administration of Vitamin A with Ferrous Sulfate in Treating Deferoxamin-Induced Iron-Deficiency Anemia

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Summary About half the pregnant women in developing countries suffer from iron-deficiency anemia. The treatment of choice for these patients includes iron compounds such as ferrous sulfate. It was recently shown that a concomitant administration of vitamin A with ferrous sulfate increases iron-induced hematopoietic effect. In the current study, the efficacy of various routes of administration of vitamin A with ferrous sulfate in deferoxamin-treated anemic rats were compared. The work reveals no difference among various routes of administration, including several alternates of oral and intramuscular injection of vitamin A and ferrous sulfate for 28 d. It was therefore concluded that the therapeutic effect of vitamin A in iron-deficiency anemia is probably not via its influence on iron absorption from the gastrointestinal tract.

Key Words anemia treatment, anemia iron deficiency, vitamin A, ferrous sulfate, deferoxamin

The importance of iron as a precursor for heme synthesis has been known for many decades (1). Iron-deficiency anemia is already a well-known entity, and it can be found alone or in association with many disease conditions. About half the pregnant women in developing countries suffer from iron-deficiency anemia.

Many reports indicate that vitamin A deficiency may also induce an anemia with a clinical picture similar to an iron-deficiency state. The anemia in these patients will be completely resolved after a course of vitamin A therapy (2, 3). Several hypotheses exist regarding the role of vitamin A in treatment of anemia, but all accept that vitamin A, through a still-obscure mechanism, enhances the iron hematopoietic effect (4–7). In this study, the effect of different routes of administration of vitamin A with ferrous sulfate in treating iron-deficiency anemia is studied.

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MATERIALS AND METHODS

Fifty-six Sprague-Dawley rats weighing 220–300 g each were maintained under well-controlled environmental and nutritional conditions for 7 d. During this period, the animals were fed by plated foods and drank water ad libitum. They were then randomly placed into eight groups of seven rats each.

After anesthesia with dichloroethan, the blood of rats in group I, through a subxyphoid approach, were taken directly from their hearts. These samples were then used for the determination of normal ranges of measured parameters. To find out the probable role of injection on the measured parameters, 1 mL of distilled water was injected intraperitoneally (IP) to each rat of group II every other day for 20 d.

The rats of the other six groups were made anemic by IP injections of 250 mg deferoxamin (Desferal) (Ciba Geigy, Switzerland), dissolved in 1 mL water, every other day for 20 d. To assess the level of deferoxamin-induced anemia 1 d after the last dose of deferoxamin, blood samples of group III rats were taken as described above. To uncover the level of recovery without any supplemental iron or vitamin A, the rats in group IV received no treatment until the end of the study, and they received only a small amount of their dietary iron and vitamin A.

Groups V to VIII were treated with vitamin A and ferrous sulfate administered by different combinations of gastrointestinal (GI) and parenteral routes of administration (Table 1). Because vitamin A is absorbed almost completely from the GI tract, its total oral (PO) and parenteral (IM) doses were selected equally (i.e., 14.3 IU/kg/d, PO, qd × 28 d ≈ 400 IU/kg, IM, stat). On the other hand, only a quarter of the orally administered ferrous sulfate is absorbed via the GI tract (8); thus the administration of 4 mg/kg/d of ferrous sulfate PO, qd × 28 d (a total of 112 mg/kg = 4 × 28 mg/kg, IM, stat = 4 × parenteral dosage).

On day 29 of the study, the blood samples of all rats (Groups IV–VIII) were taken for measurement of the red blood cell count (RBC), RBC indices [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)], hemoglobin (Hb), hematocrit (Hct), serum iron (SFe), and total iron-binding capacity (TIBC). The group means were compared by one way analysis of variance (ANOVA) and Duncan’s multiple-range test.

RESULTS

The mean ± SD of the measured variables for different groups are shown in Table 2. No difference was found among the measured variables between groups I and II. The measured hematological parameters in group III (control for anemia) significantly differed from those of other groups (*p < 0.05). The measured parameters in group IV (control for treatment), which had received no treatment during the experiment, were significantly different from those of groups I and III (*p < 0.05).
Table 1. Experimental conditions.

| Group | Administration of deferoxamin* | Ferrous sulfate (mg/kg) | Vitamin A (IU/kg) | To answer… |
|-------|-------------------------------|-------------------------|-------------------|------------|
| I     | −                             | −                       | −                 | What are the normal values? |
| II    | −                             | −                       | −                 | Does injection itself affect the measured values? |
| III   | +                             | −                       | −                 | Has deferoxamin induced anemia? |
| IV    | +                             | −                       | −                 | Have rats recovered if no treatment had been instituted? |
| V     | +                             | 4, PO, qd × 28d         | 14.3, PO, qd × 28d| The effect of different combinations of therapeutic modalities |
| VI    | +                             | 28, IM, stat            | 14.3, PO, qd × 28d| The effect of different combinations of therapeutic modalities |
| VII   | +                             | 28, IM, stat            | 400, IM, stat     | The effect of different combinations of therapeutic modalities |
| VIII  | +                             | 4, PO, qd × 28d         | 400, IM, stat     | The effect of different combinations of therapeutic modalities |

IP: intraperitoneally; qd: every day; qod: every other day; IM: intramuscular; stat: single dose; IU: international unit; kg: kilogram.

* 250 mg, IP, qod, 20 d.
Table 2. Changes in various parameters after treatment with ferrous sulfate and vitamin A.

| Group | RBC (10^6/μL) | Hb (g/dL) | Hct (%) | MCV (FL) | MCH (pg) | MCHC (g/dL) | SFe (g/dL) | TIBC (g/dL) |
|-------|---------------|-----------|---------|----------|----------|-------------|------------|------------|
| I     | 9.80 ± 0.30   | 16.32 ± 0.77 | 45.07 ± 1.52 | 45.95 ± 0.41 | 45.74 ± 0.30 | 36.19 ± 0.61 | 36.19 ± 0.61 | 268.82 ± 8.26 |
| II    | 9.82 ± 0.17   | 16.42 ± 0.41 | 45.96 ± 1.09 | 45.74 ± 1.84 | 32.40 ± 0.10 | 29.70 ± 0.87 | 93.28 ± 6.14 | 33.28 ± 23.58 |
| III   | 7.39 ± 0.32   | 7.12 ± 0.55 | 23.16 ± 1.14 | 36.59 ± 0.85 | 11.36 ± 1.09 | 31.51 ± 0.90 | 115.36 ± 5.93 | 846.60 ± 29.82 |
| IV    | 8.40 ± 0.30   | 9.64 ± 0.52 | 30.59 ± 1.76 | 36.59 ± 1.84 | 16.26 ± 0.63 | 36.33 ± 0.59 | 234.89 ± 9.94 | 90.32 ± 25.17 |
| V     | 9.18 ± 0.61   | 15.14 ± 0.57 | 42.23 ± 2.58 | 36.59 ± 1.84 | 16.26 ± 0.63 | 36.33 ± 0.59 | 234.89 ± 9.94 | 590.44 ± 25.22 |
| VI    | 9.31 ± 0.57   | 15.14 ± 1.05 | 42.23 ± 2.58 | 45.46 ± 0.36 | 36.22 ± 1.02 | 36.22 ± 1.02 | 234.89 ± 9.94 | 583.30 ± 18.29 |
| VII   | 9.23 ± 0.32   | 15.23 ± 0.66 | 42.03 ± 1.00 | 45.46 ± 0.36 | 36.22 ± 1.02 | 36.22 ± 1.02 | 234.89 ± 9.94 | 588.85 ± 27.23 |
| VIII  | 9.26 ± 0.40   | 15.29 ± 0.66 | 42.43 ± 1.00 | 45.83 ± 0.60 | 16.59 ± 0.24 | 36.24 ± 0.44 | 236.47 ± 26.25 | 585.67 ± 26.25 |

RBC: red blood cell count; Hb: hemoglobin; Hct: hematocrit; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; SFe: serum iron; TIBC: total iron-binding capacity.

Mean ± SD that are not significantly different have the same superscript.
The measured hematological parameters of the four treatment groups (V–VIII) were returned to their normal values; nevertheless, they significantly differed from those of groups I (normal), III (control for anemia), and IV (control for treatment) ($p<0.05$).

DISCUSSION

Anemia is one of the commonest clinical findings and may be associated with many disease conditions. Most anemic states are caused by malnutrition, especially insufficient iron, vitamin B$_{12}$, and copper (1, 9). It was recently shown that vitamin A deficiency may also lead to anemia (10), and vitamin A was found to have an important role in iron metabolism (7, 11).

One study showed that vitamin A supplementation produces a significant rise in plasma retinol level, Hb, Hct, RBC count, and SFe, and it enhances iron metabolism (4). Another article to reveal the synergistic effect of vitamin A and iron on hematopoiesis proposed a probable mechanism, i.e., the involvement of vitamin A in the regulation of hepatic iron release (6). In a previous study, the coadministration of vitamin A and iron in 63 iron-deficient anemic pregnant women who had had no vitamin A deficiency significantly increased their blood Hb level, a third of which could be attributed to the vitamin A administration and two thirds to the iron supplementation (7). In that work, however, the mechanism by which vitamin A enhances iron metabolism was not declared.

Several hypotheses explaining the role of vitamin A have already been proposed (5, 6, 11, 12). Although an increase was noted in the liver and spleen iron stores of some rats deprived of vitamin A that had had enough iron intake, the rats developed iron-deficiency anemia after a short time (5, 6, 8). This indicates the probable role of vitamin A in iron transfer to its tissue stores. Some researchers believe that vitamin A inhibits the entrance of iron into bone, thus increasing the SFe that in turn augments the liver and spleen iron stores (5). Some also advocate that vitamin A deficiency decreases the bone marrow iron uptake, which is probably due to an inhibition of RBC iron binding that results in decreased hematopoiesis (11).

Because of the very large pile of data (7, 13–16) in this study, no effort was made to reexamine the synergistic effect of vitamin A and iron on hematopoiesis; thus no group was treated with either vitamin A or ferrous sulfate alone. For the same reason, serum and liver vitamin A levels were also not measured.

The similarity of measured parameters in groups I (normal) and II (control for injection) indicates that the injection itself is not responsible for the difference observed among the other groups.

In this study, for the induction of iron-deficiency anemia, deferoxamin (Desferal), a chelating agent derived from Streptomyces pilosus, was used (8). Deferoxamin was completely successful in the induction of anemia, as reflected by the significant difference between groups I (normal) and III (control for anemia) ($p<0.01$). The rats in the treatment control group (IV) that have received no
treatment partially compensated their anemia; though the measured parameters in this group differed greatly from those of group III (control for anemia) \((p<0.05)\), they were not completely returned to their normal ranges. The alleviation seen in this group was perhaps due to the dietary intake of iron and vitamin A during the experiment. In treatment groups (V–VIII), regardless of the drug administration routes, though Hb, Hct, RBC, SFe, and TIBC were recovered significantly during the 28-d treatment period \((p<0.05)\), they did not reach their normal values and showed a significant difference also with those of group I (normal control) \((p<0.05)\). This is probably due to the short treatment period. MCV, MCH, and MCHC, however, were more sensitive to treatment, and their values became normal even during this short time interval of drug therapy.

Because of similar results obtained from different forms of treatment groups, it can be concluded that the route of administration of vitamin A and ferrous sulfate is not an important therapeutic factor; therefore we can state that vitamin A perhaps has no effect on iron absorption from the GI tract, and it exerts its effect by another mechanism that needs more elucidation.

REFERENCES

1) Mertz W. 1986. Trace Element Metabolism in Human and Animal Nutrition, 5th ed, Vol 1, p 79–142. Academic Press, New York.
2) Bloem MW, Wedel M, Van Agtmaal EJ, Speek AJ, Saowakontha S, Schreurs WH. 1990. Vitamin A intervention—Short term effects of a single oral massive dose on iron metabolism. Am J Clin Nutr 51: 76–79.
3) Mejia LA, Arroyave G. 1982. The effect of vitamin A. Fortification of sugar on iron metabolism in preschool children in Guatemala. Am J Clin Nutr 36: 87–93.
4) Mejia LA, Chew F. 1988. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. Am J Clin Nutr 48: 595–600.
5) Mejia LA, Hodges RE, Rucker RB. 1979. Role of vitamin A in the absorption, retention, and distribution of iron in the rat. J Nutr 109: 129–137.
6) Staab DB, Hodges RE, Metcalf WK, Smith JL. 1984. Relationship between vitamin A and iron in the liver. J Nutr 114: 840–844.
7) Suhrano D, West CE, Muhilal, Karyadi D, Hautvast JG. 1993. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet 342: 1325–1328.
8) Gilman AG, Goodman LS, Rall TW, Murad F. 1985. Goodman and Gilman’s The Pharmacological Basis of Therapeutics, 7th ed, Vol 2, p 1314–1318, 1624. Macmillan, New York.
9) Nockels CF, Kienholz EW. 1967. Influence of vitamin A deficiency on testes, bursa fabricius, adrenal gland and hematocrit in cockerels. J Nutr 67: 384–388.
10) Lee GR, Bithel TC, Foerster J, Athens JW, Lukens JN. 1993. Wintrobe’s Clinical Hematology, 9th ed, Vol 1, p 715–716, 727, 730–731. Lea and Febiger, Philadelphia.
11) Siijtsma KW, Van Den Berg GJ, Lemmens AG, West CE, Beynen A. 1993. Iron status in rats fed on diets containing marginal amounts of vitamin A. Br J Nutr 70: 777–785.
12) Mejia LA, Hodges ER, Rucker RB. 1979. Clinical signs of vitamin A deficient rats. Am J Clin Nutr 32: 1439–1444.
13) Bloem MW, Wedel M, Egger RJ, Speck AJ, Schrijver J, Saowakontha S, Schreurs WH. 1989. Iron metabolism and vitamin A deficiency in children in northeast Thailand. *Am J Clin Nutr* **50**: 332–338.

14) Hodges RE, Sauberlich HE, Canham JE, Wallace DL, Rucker RB, Mejia LA, Mohanram M. 1978. Hematopoietic studies in vitamin A deficiency. *Am J Clin Nutr* **31**: 876–885.

15) Mejia LA, Hodges RE, Arroyave G, Viteri F, Torum B. 1977. Vitamin A deficiency and anemia in central American children. *Am J Clin Nutr* **30**: 1175–1184.

16) Panth M, Shatrugna V, Yasodhara P, Sivakumar B. 1990. Effect of vitamin A supplementation on haemoglobin and vitamin A levels during pregnancy. *Br J Nutr* **64**: 351–358.