ARTIGO ORIGINAL

Iron peptide complex does not increase blood iron concentration at the same extent as ferrous sulfate after oral ingestion in healthy adult males

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ABSTRACT

Iron-deficient anemia continues to be one of the major nutritional and public health problems all over the world. Although ferrous sulfate is largely used for the prevention and treatment of this deficiency, the gastrointestinal side effects preclude its use in some cases. This study aimed to compare the effect of iron peptide complex and ferrous sulfate on serum iron and to examine serial serum iron levels. Ten volunteers were submitted to 5 different treatments: Control (C), Ferrous sulfate 60 mg (FS), Iron peptide 60 mg (IP1), Iron peptide 80 mg (IP2) and Iron peptide 60 mg plus diet (IPD). In the first treatment (C), empty capsules were given, whereas in the second (FS), third (IP1), fourth (IP2) and fifth (IPD) treatments, ferrous sulfate 60 mg, iron peptide complex 60 mg, iron peptide complex 80 mg and iron peptide complex 60 mg plus diet were given in a randomized crossover design, with a washout period of 1 week. The products were offered as capsules and blood samples were drawn at the following time points: 0, 30, 60, 120, 240, 480 and 720 minutes after oral intake. FS produced higher serum iron levels than Control, IP1, IP2 and IPD ($P < 0.05$). The areas under the curves for serum iron for the different compounds gave $AUC_{FS} > AUC_{C} = AUC_{IP1} = AUC_{IP2} = AUC_{IPD}$. Conclusions: The iron peptide complex did not increase blood iron concentration as compared to control and ferrous sulfate at the respective time points.

Key words: Ferrous sulfate, iron-peptide complex, serum iron, anemia, iron deficiency

INTRODUCTION

Iron-deficient anemia continues to be one of the major nutritional and public health problems all over the world, affecting about 2 billion people WHO (1998) [1], especially in developing countries (WHO/UNU/UNICEF 1993) [2]. These conditions are mainly due to inadequate iron ingestion.

Several substances containing non-heme iron are available for the prevention of iron deficiency, such as ferrous sulfate, ferrous gluconate, ferrous lactate, ferrous fumarate, ferrous succinate, elemental iron, ferric EDTA, glycine iron etc. Ferrous sulfate is largely used for the prevention and treatment of iron deficiency anemia due to its low cost. However, patient compliance with treatment in programs of iron supplementation with ferrous sulfate is usually poor due to side effects related to the gastrointestinal system [3,4]. In this respect, an iron-peptide complex has been developed [5] whose physicochemical characteristics contrast to those of ferrous sulfate. This is an organic complex of low solubility in acid pH and fully soluble in neutral and alkaline pH. The iron in the iron-peptide complex remains insoluble during its passage through the stomach due to the low pH. In the duodenum it becomes soluble but is expected to remain in the complexed form. Iron is released from the complex by a still unclear mechanism, to be later absorbed through the same pathway as that of other forms of non-heme iron. Although studies conducted on pregnant women [6] and on anemic rats [7] have shown that iron-deficiency anemia

could be treated and prevented by iron peptide complex, there is still controversy regarding its efficacy for iron supplementation. The recommended dose of elemental iron in iron deficiency states is 60 mg 3 times daily [8]. The rationale and justification for the present research are based on having an alternate iron supplement to treat iron deficiency anemia as efficient as ferrous sulfate but with less side effects, bringing new insights into iron deficiency anemia treatment. Therefore the objective of the present study was to measure the serum iron concentration after oral intake of iron peptide complex as compared to ferrous sulfate.

We hypothesized that iron peptide complex administered orally to healthy adult men induces increase in serum iron concentration similar to the increased induced by ferrous sulfate.

METHODS AND MATERIALS

The study was conducted on 10 healthy male volunteers aged 21 to 43 years, with a body mass index of 20 to 25 kg/m^2. All subjects were submitted to routine clinical, anthropometric, hematologic and parasitologic exams and to biochemical exams for the determination of serum iron, serum ferritin, and glycemia in order to insure the absence of physiological and pathological factors influencing blood iron concentrations.

The study was conducted on volunteers who did not perform intense physical activity for more than 2 hours/day, without diarrhea, who took no medication, who had no diseases involving the stomach, pancreas intestine, liver or bile ducts, who did not intend to donate blood during the study period, who did not present blood parameters (hemoglobin, hematocrit, red blood cells) below normal values, and who had no microcytosis or hypochromia, intestinal parasitosis, diabetes mellitus, arterial hypertension, renal insufficiency, obesity, malnutrition, smoking habit, or alcoholism..

The Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (HCFMRP-USP) (Process HCRP 10804/00) approved the study and the volunteers were selected from among the employees of the same institution who participated in the On-the-Job Gymnastics Program. All volunteers gave written informed consent to participate in the study.

Study design

Each volunteer was submitted to one of the 5 different treatments in a randomized crossover design. A one-week washout interval was used between studies to minimize intra and interindividual variability. On all first day of each treatment, after an 8-hour overnight fast the volunteers ingested the capsules containing the supplements to be tested. Venous blood was collected after supplement ingestion for 12 hours at the following time points: 0, 30, 60, 120, 240, 480 and 720 minutes. The five treatments are described bellow.

First treatment: Control (C). This treatment was used as control for comparative purposes, with the subject ingesting empty gelatin capsules at time zero with 250 ml of deionized water.

Second treatment: Ferrous sulfate (FS). The volunteers ingested capsules containing 60 mg iron in the form of SF at time zero.

Third treatment: Iron-peptide complex (IP1). The volunteers ingested capsules containing 60 mg iron in the form of IP1.

Fourth treatment: IP2. The volunteers ingested capsules containing 80 mg iron in the form of IP.

Fifth treatment: Iron-peptide complex with the diet (IPD). The volunteers ingested capsules containing 60 mg iron in the form of IP with the diet.

On the day before being tested, the volunteers were instructed to eat a diet poor in calcium and fibers, and not to eat food of animal origin, such as meat and liver. They fasted for a period of 480' overnight. For example: from 23:00 to 7:00 hours.

Blood sample collection

Three mL venous blood were collected at each time point described into transparent, colorless siliconized, sterile vacuum glass tubes containing an inert separating gel for serum and a clot activator (Becton Dickinson®, reference 367783).

Determination of blood iron concentrations

Blood iron concentrations were determined with a Cobas Integra 700 instrument (Roche®) and a spectrophotometric method employing the Ferrozine® reagent (Stookey, 1970). The mean blood iron concentrations ($\pm$ SD) obtained with each treatment were determined from individual values at the 0, 30, 60, 120, 240, 480 and 720 minutes time points. The means ($\pm$ SD) of the increase in blood iron concentrations were calculated as the differences between the concentrations determined at each time point and the concentrations at time 0 for the C, FS, IP1, IP2 and IPD. The results obtained were analyzed by comparing the increase in control blood iron concentrations to those obtained after the different treatments (SF, IP1, IP2 and IPD). The increases in blood iron concentrations obtained after the administration of ferrous sulfate were then compared to those obtained after the IP1, IP2 and IPD treatments.

Determination of the areas under the curves for the C, FS, IP1, IP2 and IPD treatments

The areas under the curves were determined using the increases in blood iron concentrations for the C, SF, IP1, IP2 and IPD treatments by the method of Matthews et al. (1990), using the following formula:

$$A = \tfrac{1}{2} \sum_{i=0}^{-1} (T_{i+1} - T_i)(Y_i + Y_{i+1})$$

where A = area under the curve (µg/dL/time in minutes)
T = time, minutes
Y = increase in blood iron concentration in µg/dL (determined by the difference between the blood iron concentrations obtained at each time point and those obtained at time 0).

In the present study, to permit an analysis without the interference of these variations, we determined the differences between control blood iron concentrations and the concentrations obtained with the various treatments in volunteers who received iron in the form of ferrous sulfate and iron-peptide complex.

In order to determine the increases in blood iron concentrations and the duration of these increases after the administration of the iron-peptide complex and of ferrous sulfate and to compare them with control concentrations, we conducted a longitudinal study over a period of 720'.

Preparation of the iron-peptide complex

The iron-peptide complex was prepared by the method of Chaud et al [6] and was administered to the volunteers in the form of a gelatin capsule

Statistical analysis

Data were analyzed statistically by nonparametric analysis of variance using the Friedman test in order to compare the C, SF, IP1, IP2 and IPD treatments in terms of the increases in blood iron concentrations determined at the different time points. The level of significance was set at 0.05. The Dunn test was used to identify the differences [9,10]. The areas under the curves for the time intervals of 0'–240', 240'–720' and 0'–720', calculated from the increase in blood iron concentrations, were also compared. The analyses were carried out using the Prism Version 3.0, Windows 95 and NT software (1999).

Results

The mean age of the subjects was 31.8 ± 7,2 years, their mean body mass index was 23.9 ± 1.7 kg/m^2 and their mean hemoglobin was 15.3 ± 0.8 g/dL. The most important findings of the present study is that the iron peptide complex, independently of the dose, did not lead to higher increases in serum iron levels as compared to ferrous sulfate. The mean serum iron level after oral intake of ferrous sulfate (129 ± 20 ug/dL) was statistically different from serum iron levels after oral intake of: IP1 (111.4 ± 18.7 ug/dL) ($p < 0.01$), IP2 (114.6 ± 23.3 ug/dL) ($p < 0.01$) and IPD (102.7 ±11.9 ug/dL) ($p < 0.01$). Statistically significant differences were also observed between mean serum iron levels after oral intake of IP1 versus IPD diet ($p < 0.01$) and after oral intake of IP2 versus IPD ($p < 0.01$) (Table 1).

The means and standard deviations of the increases in blood iron concentrations as a percentage of dose, obtained after the C, SF, IP1, IP2 and IPD treatments are presented in Figure 1. The areas under the curve from time zero to 240 minutes were significantly different between FS and C ($p < 0.01$) and FS and IP1 ($p < 0.05$). From time 240 to 720 minutes there were significant differences between FS and IP1 and FS and IPD ($p < 0.05$) (Table 2).

DISCUSSION

Serial changes in plasma iron concentrations in 10 healthy adult male volunteers after the ingestion of specified doses of an iron-peptide complex and ferrous sulfate were studied.

Ferrous sulfate is considered to be a reference compound due to its high bioavailability compared to other compounds [11,12] and is therefore used in studies aiming at the evaluation of other substances containing iron [13-17].

Statistical analysis of the accumulated areas under the curves suggested that the initial effects of the iron supply would be observed during the first 240', whereas determination of the area obtained after the 240'–720' interval permitted the analysis of the late effects of iron ingestion. The 240' time point was established for the purpose of analysis on the basis of previous studies that confirmed the increase in blood iron concentration up to this time. The results obtained after the 0'–240' interval showed increases in the areas under the curve due to the administration of ferrous sulfate compared to control areas, suggesting that iron was absorbed, with a consequent increase in blood iron concentration. No increase in the areas ($p > 0.05$) was observed during this period after the administration of the iron-peptide complex in the IP1, IP2 and IPD treatments when compared to control.

The higher level of significance observed when

the areas of the FS versus C were compared ($p < 0.01$) to FSxIP1 ($p < 0.05$) may have been due to low iron absorption when the iron-peptide complex was administered under the same conditions. The lack of a difference ($p > 0.05$) between the areas for the FS versus IP2 and FS versus IPD treatments may have been due to greater iron absorption, although still small, when the iron-peptide complex is administered in a quantity equivalent to 80 mg iron under fasting conditions, or to 60 mg with the diet, compared to the administration of the equivalent of 60 mg iron in the form of ferrous sulfate under fasting conditions.

The reduction observed in all volunteers at 30' in relation to time zero, in all treatments, may have possibly been due to the hemodilution caused by the ingestion of 250 mL deionized water.

Comparison of the 0'-240' period to the other time points regarding the increase in blood iron concentration demonstrated a significant difference ($p<0.01$) between the SF and C treatments at 60', 120' and 240', confirming the results obtained in the analysis of the accumulated areas under the curves. Differences between the SF and IP1 treatments at 120' and 240' indicated that the supposed absorption due to the administration of 60 mg iron as an iron-peptide complex may have started 120' after administration. The differences observed at 240' and 480' may suggest that the supposed absorption may be initially favored during the first two hours, decreasing thereafter. There was no significant difference between SF and IP2 at any time point studied when the increase in blood iron concentration was compared, probably due to greater iron absorption compared to the administration of an iron-peptide complex equivalent to 60 mg iron.

The previously presented hypothesis related to the absorption of iron administered to non-deficient individuals in the form of an iron-peptide complex, which was low when compared to that observed after the administration of ferrous sulfate, could be elucidated only by administering an iron-peptide complex prepared with stable iron isotopes.

After administering ferrous sulfate in a quantity equivalent to 120 mg iron, i.e., twice the amount used by us, Silva et al. [18] obtained a blood iron concentration profile similar to that obtained in the present study. They also detected a mean increase in blood iron concentration of 118.5 µg/dL at the 240' time point, a value higher than that detected in the present study, which was 51 µg/dL. These values suggest that the absorption of iron administered in the form of ferrous sulfate is dose dependent and is little controlled by the levels of stores in the organism. On the other hand, we cannot exclude intermediate peaks of blood iron concentrations, which were not detected with the methods used, although this is an unlikely possibility.

The method lacks sensitivity because of the choice of adult men, whose iron requirements and absorption rates would be expected to be low. The failure to detect significant increases in plasma iron concentration may in part be due to the low sensitivity of the experimental model. However, this model was used in order to provide results that could be further applied in individuals with iron deficiency. In individuals with iron deficiency, the increase in blood iron concentration would have probably been higher than that observed in the present study due to the physiological mechanisms of control of iron absorption. Non-absorbed iron may possibly be the cause of the problems involved in the use of ferrous sulfate such as diarrhea, constipation, modifications of the fecal flora [19], the increase in free radicals in the feces [20] and damage to the intestinal mucosa.

Under conditions of acute overdosage, absorption is assumed to occur by a first-order passive process [21]. This increase in blood iron concentration not controlled by the organism may result in iron accumulation, a fact that precludes the use of ferrous sulfate in supplementation programs at the population level. The absorption of iron in the form of a complex may be better regulated by iron stores in the organism [14, 22, 23]

The results observed after the 240'–720' interval demonstrated that there was no significant difference between the areas of control blood iron concentration and the concentrations determined after the SF, IP1, IP2 and IPD treatments. This suggests that the iron absorbed during the initial 240' after the administration of ferrous sulfate, which may be bound to apotransferrin, was partially transferred to other compartments and that during this time interval, corresponding to its passage into the large bowel, iron did not continue to be absorbed, in contrast to the observations of Cook et al [24].

The IP1, IP2 and IPD treatments did not show significant differences compared to control at any time point evaluated, suggesting that, when administered under fasting conditions to healthy men, followed by a diet free from iron absorption inhibitors up to 720' after administration, the iron-peptide complex does not induce or favor a significant increase in blood iron concentrations.

The significant differences at the 120' 240' and 480' time points, between increases in blood iron concentrations induced by the administration of ferrous sulfate and of the iron-peptide complex under the same conditions revealed significant differences demonstrating that, when administered to healthy men, ferrous sulfate causes a significantly higher increase in blood iron concentration compared to the iron-peptide complex.

CONCLUSION

The iron-peptide complex administered orally under fasting conditions in a quantity equivalent to 60 or 80 mg iron induces significantly lower increases in blood iron concentration than the administration of 60 mg ferrous sulfates at the time points studied.

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