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CALCIUM CARBONATE REDUCES IRON ABSORPTION FROM IRON SULFATE, BUT NOT WHEN IRON IS PRESENTED AS AN ORGANIC COMPLEX

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ABSTRACT: Experimental and epidemiological evidences have demonstrated that calcium inhibits iron absorption; calcium carbonate being one of the most effective calcium sources to reduce iron absorption from dietary origin or from iron sulfate. In the present work, the short-term effect of calcium from calcium carbonate on iron absorption was studied in rats, using different iron compounds (monosodium ferric EDTA, iron-bys-glicine, iron peptide complex) with iron sulfate as a control. Eighty (80) animals were divided into groups of 10 animals each with homogeneous weight. After 18h fast, the animals received by gavage 5 mL of a dispersion containing one of the iron compounds (1mg Fe/kg body weight), concomitantly or not with calcium carbonate at a molar ratio of 150:1 (Ca/Fe). Two hours after the administration, the animals were sacrificed and blood was collected for serum iron determination (iron transfer rate from intestinal lumen to blood compartment). Additionally, the intestines were collected for soluble iron determination (available iron). The results demonstrated that calcium ion from calcium carbonate inhibits the iron absorption from iron sulfate, but not from organic iron (di- or trivalent) complexes.

KEYWORDS: Iron absorption; iron complexes; calcium.

Introduction

Studies in animals and humans have demonstrated that calcium salts reduce the heme and non-heme iron absorption and that the extent of this effect was dose dependent[18]. Calcium carbonate, the most common calcium salt used in supplements[17], reduced the iron bioavailability and hemoglobin regeneration to a greater extent than calcium sulfate did[21]. Similar results were reported in studies on human beings using a molar ratio of Ca/Fe varying from 88:1 to 281:1[7, 20]. Most reports in the literature have studied the effect of different calcium salts, their relative concentration and route of administration on heme and non-heme iron bioavailability. In non-heme iron studies, the main iron sources were food, which could contain inhibitors or promoters of iron absorption[5], or inorganic salts, especially iron sulfate[1]. In addition, the elderly, who very often are iron and calcium deficient, use formulas consisting of dietary supplements[14]. Over the counter formulations for oral or enteral nutritional support are available on the market and vary widely in form of nutrients, presentation and in relative composition.However, all formulations contain nitrogen, carbohydrates, lipids, vitamins, and minerals. Among these minerals are calcium and iron at different Ca/Fe molar ratios[7, 8]. Based on these considerations, and on the lack of studies using alternative iron sources, the objective of the present investigation was to assess in rats the short-term effect of calcium carbonate on iron absorption from organic iron complexes, when co-administered in a dispersion containing the macronutrients of a chemically defined oral/enteral diet.

Materials and methods

Materials

Iron sulfate heptahydrate (19.3% of Fe^{+2}), was from Merck, Germany; monosodium ferric EDTA, EDTA-Fe^{+3} (13.3% of Fe^{+3}), from Dr. Paul Lohmann, Germany; iron-bys-glycine complex, Fe^{+2}-Gly (18.4% of Fe^{+2}), from Albion Laboratories Inc., USA; iron peptide complex, Fe^{+3}-peptide (4.0% of Fe^{+3}), was prepared in our laboratory, as described by Chaud et al[2]. Calcium carbonate (99% of Ca^{+2}), was from Merck, Germany; casein (85.0% of protein), was from Katuffmann & Co., Germany; maltodextrin (Mor-Rex®1920), from Corn Products S.A., Brasil; corn oil (Mileto®), from Ceval Alimentos S.A., Brasil; kit for serum iron determination was from Companhia Equipadora de Laboratórios Modernos, CELM, Brasil). All the other

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reagents used were of analytical grade.

Animals

Rats were obtained from the animal house of the Ribeirão Preto Campus, University of São Paulo, and were maintained in accordance with the Guide for the Care and Use of Laboratory Animals[19]. Male Wistar rats (170 to 225 g), with hemoglobin levels ≥12μg/dL[6] were housed in individual stainless-steel metabolic cages in a room with a 12h light/dark cycle, at 25ºC±1ºC. The animals were used in the experiments after a 3 days adaptation period. In all experiments, animals were dosed by gastric gavage, in a volume of less than 5% their body weight. Some animals died during the experiment due to wrong administration of gavage.

Preparation of the dispersion containing iron and calcium

The iron compounds and/or calcium carbonate were administered in a dispersion consisting of protein (10%), carbohydrate (80%), and lipid (10%). The final dispersion was planned to contain calcium (from calcium carbonate) and iron (from iron sulfate, EDTA-Fe^{+3}, Fe^{+2}-gly or Fe^{+3}-peptide) at a Ca/Fe molar ratio of 150:1 in 5 mL, and a dose of 1 mg Fe/kg body weight.

Experimental procedure

After 18h fasting, with free access to physiological glucose solution, the animals were divided into eight groups with ten animals each with homogeneous weight. Half of the groups received, by gastric gavage 5 mL of a dispersion containing one of the iron compounds, and the other half received 5 mL of the dispersion containing both the iron compound and calcium carbonate at the molar ratio indicated above. Two hours after administration, blood was collected by cardiac puncture, under superficial ether anesthesia and animals were sacrificed. Serum was separated and used for serum iron determination. The intestines were removed and their lumens carefully washed with cold NaCl solution (0.9%, w/v). The volume collected from the internal intestinal washings was made up to 13 mL, and centrifuged. The supernatant was used to quantification of soluble iron by atomic absorption spectroscopy (Shimadzu instrument model AA 680G). Serum iron was determined by a colorimetric method using ferrozine, pyridyl-phenyl sulfonic acid triazine[13] at 560 nm in a Beckman DU 640 spectrophotometer.

Data were analyzed statistically by one-way analysis of variance (Anova). When significant effects of calcium were found, differences between means were assessed by the post-hoc Duncan test (significance level of $p<0.05$), using the software Statistica for Windows, version 4.5 (Statsoft, Inc., 1993).

Results and discussion

No technique or animal model is totally suitable for the study of bioavailability of nutritional or therapeutic formulations. The rat is limited as a model for human beings due to differences in feeding behavior, consumption of energy by body area and heme iron absorption[6]. However, the results obtained from parallel studies of mineral metabolism in humans and rats are usually consistent[11]. In the present study, we assumed that the soluble iron quantified in the washing from intestinal lumen can be considered as available iron; the elevation of serum iron levels after oral administration to intact rats under normal conditions was considered to be an index of iron transfer from the intestinal lumen to the blood stream. This transfer includes the iron absorption by enterocytes, cytoplasmic transit and the true transfer to the blood compartment. The presence of calcium carbonate reduced iron availability (soluble iron in the intestinal compartment) in the group that received iron sulfate iron; the group that received EDTA-Fe^{+3} plus calcium presented an increase of iron availability, and the other groups presented no significant differences (Table 1). The iron transfer from the luminal compartment to the blood was reduced in the group that received iron sulfate plus calcium carbonate, when compared to the group that received only iron sulfate ($p<0.001$, Table 1). Our result corroborates those reported by Prather & Miller[21] and Kim & Atallah[15].

In the present study, the correlation between the increase of soluble iron in the intestine and the increase of serum iron[16] occurred only in the group that received iron sulfate. In the group that received EDTA-Fe^{+3}, the presence of calcium carbonate increased significantly the content of soluble iron, but produced no alterations in serum iron levels. In all other groups, the presence of calcium induced no alterations in serum iron levels (Table 1).

The differences of availability and bioavailability among the iron sources could be partially explained by chemical speciation of iron during gastrointestinal transit. A complex group of chemical species of Fe^{+2} and Fe^{+3} is produced because of the pH variation from the stomach ($pH \leq 2.0$) to the intestine ($6.0 < pH > 8.0$)[22]. The ferrous salts are weakly coordinated anions ($FeSO_4.6H_2O$) which can generate Fe^{+2} hydrate – $Fe(H_2O)_6^{+2}$ – in gastric pH. In the intestinal pH, it suffers oxidation to ferric form and hydrolysis[9]. Ferric salts like $FeCl_3 6H_2O$ originate Fe^{+3} hydrate – $Fe(H_2O)_6^{+3}$ – in $pH \leq 1$. Fe^{+3} hydrate, originating from both ferrous and ferric salts, at acid pH (1-2) suffers hydrolysis to ferric hydroxide, $Fe(OH)_3$, and/or polymerization, thus forming larger insoluble polynuclear species[22]. The iron salts (ferrous or ferric) in the intestinal lumen are present as hydroxides, and their absorption depends on their solubility[10]. More stable complexes as ferritin and transferrin, that suffer little or no dissociation in the stomach, reach the intestines predominantly in the soluble form, therefore available for absorption[23]. In the present study, the lack of effect on iron bioavailability when

Table 1 - Effect of concomitant administration of calcium carbonate and different iron sources on soluble iron in intestine and on serum iron levels, after two hours of administration

Iron sources	Calcium	Soluble iron in intestine μg/13mL	Serum iron μg/13dL
$Fe^{+2}SO_4$	-	92.34±6.55 (n=10)[a]	410.27±29.27 (n=10)[a]
	+	34.00±2.44 (n=6)[b]	264.44±16.54 (n=10)[b]
EDTA-Fe^{+3}	-	14.00±2.20 (n=10)[a]	282.45±21.28 (n=8)[a]
	+	71.17±6.10 (n=10)[b]	265.57±19.53 (n=10)[a]
Fe^{+2}-Gly	-	8.37±1.11 (n=7)[a]	340.11±23.58 (n=9)[a]
	+	13.72±3.13 (n=7)[a]	380.78±36.59 (n=8)[a]
Complex Fe^{+3}-peptíde	-	11.04±2.34 (n=7)[a]	394.28±23.61 (n=9)[a]
	+	23.25±3.48 (n=5)[a]	387.98±42.99 (n=8)[a]

The data are presented as mean ± SEM. The numbers in parenthesis indicate the number of animals in each group. Different letters indicate statistical difference ($p \leq 0.001$) when calcium carbonate was co-administered by gastric gavage with iron compound in a 5mL dispersion.

calcium carbonate was co-administered with iron complexes (EDTA-Fe^{+3}, Fe^{+2}-gly, and Fe^{+3}-peptide) could be attributed to its higher stability and solubility during its gastrointestinal transit. The differences in solubility of Fe^{+3}-peptide complex and iron sulfate deserve a special comment. Iron sulfate is soluble in acid pH, while the Fe^{+3}-peptide complex is insoluble. At neutral pH, iron sulfate is insoluble, and the Fe^{+3}-peptide complex is insoluble, but the iron stays in the complex form[2].

Another hypothesis to explain the difference between inorganic and complex iron is based on the iron absorption mechanism. A divalent cation transporter denominated DMT-1 (divalent metal transporter, or Nramp2) mediates the non-heme iron absorption through the apical membrane of the enterocyte[4, 12]. The divalent iron is more efficiently transported by DMT-1 than the trivalent iron; however, both could be absorbed by enterocyte through other pathways. One of these pathways is the 520 kDa paraferritin complex (beta-integrin, mobilferrin and flavin monoxidase) that participates in the iron absorption mediated by mucin in the intestinal lumen[24]. The mechanism of iron absorption for this pathway has not been completely elucidated. However, the most probable events are that the trivalent iron is solubilized by mucin, mobilized by the paraferritin complex and then internalized[3]. Once absorbed, Fe^{+3} suffers reduction mediated by flavin monoxidase in the cytoplasm. Fe^{+3} is absorbed through paraferritin, while Fe^{+2} is absorbed through DMT-1, and zinc or copper does not share the two absorption mechanisms, while magnesium shares the mechanism of Fe^{+2} absorption, without affecting the Fe^{+3} absorption[12].

In the present study, when we compared the relative values of iron sulfate (Fe^{+2}) to those of the Fe^{+3} complexes, the different absorption mechanisms for Fe^{+2} and Fe^{+3} could explain partially our results. Calcium is a divalent metal with similar characteristics to magnesium and may compete with iron in binding to the transporter. However, these mechanisms could not explain the behavior of iron from Fe^{+2} from Fe^{+2}-gly.

Regarding the effect of calcium of iron absorption, one should consider: a) the form of iron presentation, as well as the nature of the ligand or complexing agent, since the binding constant will determine the alterations that iron can suffer along the gastrointestinal tract, and consequently its solubility (availability), and b) the mechanisms described above for the iron absorption suggest different affinities for Fe^{+2} and Fe^{+3}, explained by the difference in availability. Independent of the mechanisms, our results suggest that whenever calcium and iron salts are associated in the same formulation, especially those destined to oral/enteral use, iron should be presented in the convenient complexed form.

CONCEIÇÃO, E.C.; MACHADO, A.A.; IZUMI, C.; FREITAS, O. Carbonato de cálcio reduz a absorção de ferro de sulfato ferroso, mas não quando o ferro é apresentado na forma de complexos orgânicos. **Alim. Nutr.**, Araraquara, v. 16, n. 1, p. 1-4, jan./mar. 2005.

■ *RESUMO*: Evidências experimentais e epidemiológicas têm demonstrado que o cálcio inibe a absorção do ferro, sendo o carbonato de cálcio uma das fontes de cálcio mais efetivas na redução da absorção do ferro de origem alimentar ou do sulfato ferroso. No presente trabalho, foi estudado em ratos o efeito agudo do cálcio (carbonato de cálcio) sobre a absorção do ferro, usando diferentes compostos de ferro (EDTA mono sódico férrico, bis-glicinato ferroso, complexo Fe^{+3}-peptídeo) tendo como controle o sulfato ferroso. Oitenta (80) animais foram divididos em grupos de 10 animais, com peso homogêneo. Após 18h de jejum, os animais receberam por gavagem, 5mL de uma dispersão contendo um dos compostos de ferro (1mg Fe/kg de peso), concomitantemente ou não com carbonato de cálcio na razão molar de 150:1 (Ca/Fe). Duas horas depois da administração, os animais foram sacrificados e o sangue foi coletado para a determinação do ferro sérico (taxa de transferência do ferro do lúmen intestinal para o

compartimento sangüíneo); adicionalmente os intestinos foram coletados para determinação do ferro solúvel (ferro disponível). Os resultados demonstraram que o cálcio oriundo do carbonato de cálcio inibe a absorção do ferro apresentado como sulfato ferroso, mas não quando o ferro (di ou trivalente) é apresentado na forma de complexo orgânico.

■ *PALAVRAS-CHAVE:* Absorção de ferro; ferro complexado; cálcio.

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