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The influence of ascorbic acid and lactose on the interaction of iron with each of cobalt and zinc during intestinal absorption

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Summary: The effect of ascorbic acid and lactose on the interaction between iron and each of zinc or copper during intestinal absorption was studied in normal and iron-deficient rats.

It was found that addition of cobalt to the iron dose inhibited absorption of iron to 42 % in normal rats and to 46.7 % in iron deficient ones. The presence of zinc with the iron dose also inhibited absorption of iron to 34.6 % in normal rats and to 32.2 % in case of the iron deficient ones.

The addition of ascorbic acid to the combined dose of Fe + Co enhanced absorption of iron by five times in normal and in iron deficient rats. In case of the combined dose of Fe + Zn the enhancement was four times in normal rats and six times in iron deficient ones.

The addition of lactose to the combined dose of either Fe + Co or Fe + Zn corrected the inhibiting action of either cobalt or zinc on iron absorption.

Based on these findings, it is recommended that ascorbic acid and lactose be added to therapeutic multimineral preparations.

Zusammenfassung: Der Einfluß von Ascorbinsäure und Lactose auf die Interaktion zwischen Eisen und Zink oder Kupfer bei der intestinalen Resorption wurde an normalen Ratten und Ratten mit Eisenmangel untersucht.

Kobalt hemmt die Resorption von Eisen bei normalen Ratten zu 42 % und bei Eisenmangelratten zu 46,7 %. Zink hemmte die Eisenresorption zu 34,6 % bzw. 32,2 %. Zusatz von Ascorbinsäure zu einer Kombination von Eisen und Kobalt steigerte die Eisenresorption bei normalen und bei Mangeltieren um das Fünffache. Bei einer Kombination von Eisen und Zink steigerte Ascorbinsäurezusatz die Eisenresorption bei normalen Tieren vierfach und bei Eisenmangeltieren sechsfach. Zusatz von Lactose zu den Kombinationen Eisen + Zink oder Eisen + Kobalt normalisierte in beiden Fällen die Eisenresorption.

Aufgrund dieser Befunde wird vorgeschlagen, Multimineralstoffpräparaten Ascorbinsäure und Lactose zuzusetzen.

Key words: iron absorption; zinc; cobalt; ascorbic acid; lactose

Schlüsselwörter: Eisenresorption, Zink, Kobalt, Ascorbinsäure, Lactose

Introduction

Iron deficiency anemia is one of the major nutritional deficiencies that affect a large sector of the population in Egypt [1, 2] as well as different

parts of the world [3]. Although the quantity of iron present in cereal based food consumed in developing countries may be satisfactory, yet the bioavailability of this iron is in most cases considerably low. The bioavailability of iron is influenced by the presence of factors that promote its absorption such as ascorbic acid or reducing sugars [4, 5], also of inhibiting factors such as phytates [6], tannins, or phosphates [7]. In addition, the absorption of iron is affected by the existence of other metals that compete with iron during absorption [8]. The presence of these metals in food is unavoidable since they are essential for normal metabolism. Therefore, it seems useful to minimize their inhibitory effect on iron absorption.

In the present investigation the effect of reducing agents such as ascorbic acid and lactose on the interaction between iron and each of zinc and cobalt during absorption was studied.

Material and Methods

White albino rats (Sprague-Dawley), body weight 150–180 g, comprising both sexes were used in these experiments.

Measurement of iron absorption was done in tied-off intestinal loops (jejunal) of rats in vivo. The rats after an overnight fast, were slightly anesthetized with diethyl ether. The abdomen was opened, and the tested dose was injected in the tied-off jejunal segment (20 cm, starting from the flexura duodeno-jejunalis). Rats were kept for 10 minutes at room temperature. Blood was then drained by heart puncture and the liver was separated. The intestinal segment was then removed and the lumen washed by perfusion, twice, with 20 ml isotonic saline phosphate buffer pH 7.4, to drain all ^{59}Fe not attached to mucosal surface.

The ^{59}Fe activity in each of blood, liver, intestinal segment and the rest of the body was measured, and corrected for the background. Measurement was done, using a γ-counter (Nuclear Enterprice SR 5).

The given dose was prepared in saline-HCl, 0.01 N solution pH 2, containing 500 nmol iron as $Fe\,Cl_3 \cdot 6\,H_2O$ labelled with ^{59}Fe obtained from (Radio Chemical Centre Ltd, Amersham, England).

The dose measured about 100,000 cpm within the standard calibration of the measuring instrument.

Each of cobalt and zinc were added to the iron dose in a concentration of 500 nmol/dose.

Experiments were done on both normal and Fe-deficient rats. Fe deficiency was produced in animals by bleeding from the tongue vein (2 ml blood, 5 times during 2 weeks). Rats were kept on an iron-deficient diet during that period [5].

Blood hemoglobin concentration was estimated following the cyanomethoglobin method described by Betke [10].

Statistical analysis of the results were made according to the Student's "t" test. Significance was assumed at $P < 0.05$.

Results

As shown in Tables 1 and 2, the addition of either cobalt or zinc to the iron dose, in equimolar ratio, caused a marked drop in iron absorption. In normal rats (Table 1), iron absorption dropped to 42 %, due to addition of cobalt and to 34.6 % in case of addition of zinc. In iron deficient rats (Table 2) the drop was 46.7 % and 32.2 % for cobalt and zinc, respectively. The iron content in the intestinal segment (Table 3 & 4) was more or less

Table 1. Whole body ^{59}Fe content (cpm) of normal rats, 10 minutes after the administration of the test dose in tied-off intestinal segments in vivo.

Dose	No addition	With ascorbic acid	With lactose
Fe	11,051 ± 593	24,972 ± 1,389 P_1 0.01	6,746 ± 1,320 P_1 0.01
Fe + Co	4,669 ± 524 P_1 0.01	25,936 ± 2,281 P_1 0.01 P_2 0.01	11,456 ± 1,004 m. s. P_2 0.01
Fe + Zn	3,848 ± 366 P_1 0.01	14,649 ± 811 P_1 0.01 P_2 0.01	11,843 ± 760 n. s. P_2 0.01

P_1 = Significance from Fe alone
P_2 = Significance from Fe + Co or Fe + Zn
P 0.01 = highly significant

not changed due to addition of cobalt to the iron dose either in normal or in iron-deficient rats. Addition of zinc to the iron dose caused an increase in ^{59}Fe content in the intestine of normal rats (52 %) (Table 3), but not in iron-deficient ones (Table 4).

Ascorbic acid, added to the Fe + Co dose, enhanced absorption of iron. The whole body uptake of ^{59}Fe in normal rats was 5 times that of rats given Fe + Co and more than twice that of rats given iron alone. In iron-deficient rats similar effect was noticed (Table 2).

Addition of ascorbic acid to the Fe + Zn dose, caused an enhancement of iron absorption more than 4 times. The whole body content of ^{59}Fe in normal rats was significantly higher than that of rats given iron alone.

In iron-deficient rats, the enhancement of iron absorption due addition of ascorbic acid to the Fe + Zn dose was more than 6 times and about double the value obtained from rats given iron alone.

The ^{59}Fe content in the intestine of normal or iron-deficient rats given ascorbic acid with the Fe + Co or Fe + Zn was markedly lower than that of rats given iron alone (Table 3 & 4).

Table 2. Intestinal ^{59}Fe content (cpm) of normal rats, 10 minutes after the administration of the test dose in tied-off intestinal in vivo.

Dose	No addition	With ascorbic acid	With lactose
Fe	23,533 ± 5,589	6,447 ± 392 P_1 0.01	13,084 ± 2,015 P_1 0.01
Fe + Co	23,757 ± 2,625 n. s.	9,031 ± 916 P_1 0.01 P_2 0.01	20,877 ± 1,082 n. s. n. s.
Fe + Zn	44,924 ± 2,080 P_1 0.01	5,411 ± 250 P_1 0.01 P_2 0.01	14,398 ± 2,484 P_1 0.01 P_2 0.01

Table 3. Whole body ^{59}Fe content (cpm) of iron-deficient rats, 10 minutes after administration of the test dose in tied-off intestinal segments in vivo.

Dose	No addition	With ascorbic acid	With lactose
Fe	20,979 ± 936		
Fe + Co	9,823 ± 498 P_1 0.01	39,791 ± 6,654 P_1 0.01 P_2 0.01	15,334 ± 6,389 P_1 0.05 P_2 0.02
Fe + Zn	6,766 ± 851 P_1 0.01	40,261 ± 1,898 P_1 0.01 P 0.01	6,693 ± 567 P_1 0.01 n. s.

Addition of lactose to the iron dose inhibited absorption of iron in normal rats. This was associated with marked drop of intestinal ^{59}Fe content.

The retardation of iron absorption caused by the addition of cobalt was completely corrected by adding lactose to the (iron + cobalt) dose in normal rats while it was partially corrected in iron-deficient ones (about 1.5 times the dose of Fe + Co), see Tables 1 & 2.

Addition of lactose to the dose of Fe + Zn corrected the inhibitory effect of Zn on iron absorption in normal rats but not in iron-deficient rats. The whole body of normal rats given Fe + Zn to which lactose was added contain remarkable amounts of ^{59}Fe which even exceeds that present in rats given iron alone (Table 1).

Discussion

The results show that the presence of either cobalt or zinc with iron in the forementioned dose levels retarded the absorption of iron in normal and iron-deficient rats. The inhibiting action of cobalt on iron absorption was previously reported by Shade et al. (1970) [11]. It has also been shown that serum iron decreased in humans given an oral dose of zinc as zinc acetate and that ferric iron in a dose of 2:1 Fe/Zn ratio reduced the plasma uptake of zinc (Solomons et al. 1983) [12]. Those authors mentioned that

Table 4. Intestinal ^{59}Fe content (cpm) of iron-deficient rats, 10 minutes after administration of the test dose in tied-off intestinal segments in vivo.

Dose	No addition	With ascorbic acid	With lactose
Fe	28,448 ± 4,225		
Fe + Co	31,678 ± 5,683 n. s.	18,670 ± 1,727 P_1 0.01 P_2 0.01	39,620 ± 2,660 P_1 0.01 P_2 0.05
Fe + Zn	27,854 ± 4,225 n. s.	13,518 ± 731 P_1 0.01 P_2 0.01	27,752 ± 1,790 n. s. n. s.

their results are most consistent with a combination of an intraluminal competition of the two minerals. In our experiments, the high ^{59}Fe content in the intestine of normal rats given Fe + Zn indicates competition between iron and zinc on the transporting agent in the intestinal mucosa, namely mucosal transferrin resulting in accumulation of iron (El-Shobaki & Rummel, 1977) [13].

Our results show that addition of ascorbic acid to these combined doses of either Fe + Co or Fe + Zn is able to stop the inhibiting effect of either Co or Zn on iron absorption. This occurred in normal and iron-deficient rats. It has been shown that addition of ascorbic acid to a solution of ferrous sulphate + zinc sulphate did not increase the inhibitory interaction of Fe on Zn, while ferric chloride showed significantly more inhibition of Zn uptake in the presence of 1 g ascorbic acid at the same ratio of Fe:Zn (Solomons et al., 1980). The inhibitory effect on zinc uptake was suggested to be dependent on the oxidation state of iron.

It seems that the presence of iron in the ferrous state prevents the competition exerted either by cobalt or zinc with iron. This is proved by the appreciable increase in iron absorption from the combined doses (Fe + Co or Fe + Zn) due to addition of the reducing agent ascorbic acid.

Lactose is a disaccharide present in milk and has also slight reducing properties. It is valuable to investigate its role on absorption and interaction of iron with either cobalt on zinc. This is important particularly to infants whose main food is milk. The presence of lactose with the combined dose of Fe + Co or Fe + Zn succeeded in correcting the inhibiting effect of either cobalt or zinc on iron absorption. The effect of lactose in this respect is less than that which occurred due to addition of ascorbic acid. It is worth mentioning that addition of lactose to the iron dose alone inhibited iron absorption. It was reported that lactose contained in milk feeds given to infants did not affect iron absorption (Ziegler & Fomov 1983) [14]. Although the reducing capacity of lactose is much less than that of ascorbic acid it has in addition chelating effects on minerals including iron. The chelated iron seems to be protected from the inhibitory action of other metals such as cobalt and zinc. The less marked effect of lactose on interaction of iron with either of cobalt or zinc in iron-deficient rats is most probably due to the enhanced rate of iron absorption in iron deficiency that mask the effect of lactose.

Although the results we obtained in this study are from short-term investigations and adaptation mechanisms may have certain implication, they represent the actual interaction of these ingredients in the gut during absorption.

On these basis we recommend to add ascorbic acid and lactose to multimineral preparations containing iron which are given to infants or adults to correct mineral deficiencies.

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