Bioavailability, Biodistribution, and Toxicity of BioZn-AAS: A New Zinc Source. Comparative Studies in Rats

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Food fortification with a proper zinc compound is an economic and effective strategy to prevent zinc deficiency. BioZn-AAS, a zinc gluconate stabilized with glycine, was compared with zinc sulfate (reference standard), zinc hydroxide, and zinc gluconate, all of them labeled with ^{65}Zn. This preclinical study was performed on Sprague-Dawley rats of both sexes, and the administered dose was 85 μg/kg of zinc. Bioavailability studies showed that absorption of BioZn-AAS was not statistically different than absorption from other sources in female rats (25.65% $\pm$ 2.20% for BioZn-AAS, 28.24% $\pm$ 4.60% for $ZnSO_4$, 24.91% $\pm$ 4.02% for $Zn[OH]_2$, and 25.51% $\pm$ 2.70% for Zn-gluconate). In the case of the male rats, absorption of BioZn-AAS (27.97% $\pm$ 4.20%) was higher ($P<0.05$) than that from the other compounds (23.15% $\pm$ 2.90% for $ZnSO_4$, 22.62% $\pm$ 3.90% for $Zn[OH]_2$, and 22.30% $\pm$ 3.90% for Zn-gluconate). Biodistribution studies demonstrated that the zinc from BioZn-AAS followed the same metabolic pathway as zinc from the other sources. Toxicity studies were performed with 50 female and 50 male rats. The value of oral lethal dose 50 (LD_{50}) was 2000 mg/kg for female rats and 1900 mg/kg for male rats. Therefore, we conclude that BioZn-AAS has adequate properties to be considered a proper zinc compound for food fortification or dietary supplementation. *Nutrition* 2000;16:762–766. ©Elsevier Science Inc. 2000

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INTRODUCTION

Zinc deficiency has become a worldwide nutritional problem that affects developed and developing countries. Several studies have shown that, independent of sex, age, and race, median intakes are about 50–80% with regard to the recommended dietary allowances for each case.[1,2]

Diet is the most important source of zinc[3] but the zinc content of food is very low,[2,4] and many nutritional factors adversely affect its absorption.[5–7] Zinc absorption takes place through the small intestine. Intestinal metallothionein holds part of the absorbed zinc in storage. The rest, transported by albumin in blood, is stored bound to the hepatic metallothionein in the liver or participates in a wide range of metabolic functions in many tissues, especially in the pancreas.[8] Bile and gastric and pancreatic secretions are responsible for zinc excretion, and once in the intestine this endogenous zinc behaves just like the dietary zinc.[5,9]

Oral acute toxicity data for humans, rats, and mice[10] of the zinc compounds used most often as dietary supplements have shown that the amount of zinc that provokes toxic effects is much higher than that contained in regular diets and that proposed in the corresponding recommended daily allowance.[5] In this way, an economic food-fortification resource seems to be a safe strategy to prevent zinc deficiency.[11]

At present, the zinc compounds used for food fortification are zinc oxide and zinc sulfate,[12] but their use has several disadvantages. Zinc sulfate modifies food sensorial characteristics, rendering the flavor of food unpalatable. Zinc oxide is poorly absorbed,[13] and it precipitates in the nutritional matrix when zinc oxide is used to fortify liquid foods. BioZn-AAS is a new alternative for food fortification, with desirable properties such as high solubility, soft taste, and not modifying the sensorial characteristics of the food. This new zinc source is a zinc gluconate stabilized with glycine. The purpose of this study was to determine its bioavailability, biodistribution, and toxicity. Bioavailability and biodistribution studies were performed to compare zinc sulfate, the reference standard, with zinc hydroxide and zinc gluconate, all of which were labeled with ^{65}Zn.

MATERIALS AND METHODS

Animals

We used 40 female and 40 male 2-mo-old inbred Sprague-Dawley rats (Radioisotope Laboratory, School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina). The female rats weighed between 140 and 200 g and the male rats weighed between 150 and 260 g. The rats were assigned to four groups, with 10 female and 10 male rats per group; rats received ^{65}Zn-

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sulfate, ^{65}Zn-hydroxide, ^{65}Zn-gluconate, or BioZn-AAS labeled with ^{65}Zn. Each group of animals was maintained in stainless-steel cages, of 315 mm × 445 mm × 240 mm high, with a stainless-steel grated floor and collection trays of the same material, thus preventing the feces from coming into contact with the animals and thus avoiding coprophagy. They had free access to deionized water and were nourished with a standard diet (Nutrimentos Diet 3, Izaguirre, Buenos Aires, Argentina). The animals were maintained with 12-h cycles of light and darkness throughout the experiment.

Synthesis of the Products

The dose of zinc administered to each rat was 85 μg/kg and represented 40% of the recommended daily allowance of a male adult human.[5] This dose was administered in water solution at a final volume of 1 mL per rat. All compounds were intrinsically labeled with ^{65}Zn except $ZnSO_4$, which was labeled by isotopic exchange by leaving the preparation overnight. The activity administered to each animal was 0.33 MBq of ^{65}Zn (NEN; Du Pont, catalog no. NEZ-109, Wilmington, Delaware, USA), which was in the chemical form of $ZnCl_2$ in 0.5 mol/L of HCl (specific activity = 3.83 GBq/mg, activity concentration = 0.25 GBq/mL), was used for labeling.

^{65}ZN-SULFATE. We labeled 0.425 mg of $ZnSO_4 \cdot 7H_2O$ (Fluka, Buchs, Switzerland; catalog no. 96501) by an isotopic exchange with 6.66 MBq of ^{65}Zn and completed the preparation to a final volume of 20 mL with deionized water.

^{65}ZN-HYDROXIDE. We mixed 0.529 mg of ZnO (Mallinckrodt Chemical Works, NY, USA) with 6.66 MBq of ^{65}Zn. We then added NaOH to produce the zinc hydroxide and then we added deionized water for a final volume of 20 mL.

^{65}ZN-GLUCONATE. We mixed 2.315 mg of D(+)-gluconic acid δ-lactone (Fluka; catalog no. 49120) with 0.529 mg of ZnO and 6.66 MBq of ^{65}Zn. The mixture was then heated 10 min at 50°C, after which deionized water was added, for a final volume of 20 mL.

BIOZN-AAS. The procedure was the same as the preparation of ^{65}Zn-gluconate, with the addition of 0.975 mg of glycine (Merck, Darmstadt, Germany; no. 4201). Deionized water was added, for a final volume of 20 mL.

Administration of the Compounds

Animals were deprived of food for 12 h before the administration of the compounds. The compounds were administered through a syringe coupled to a plastic gastric catheter, for a standard intake volume of 1 mL. Food was provided 4 h after the administration.

Absorption Studies

We measured the activity retained by each rat as a function of time by a gamma spectrometer with a 5-cm × 5-cm NaI (Tl) well crystal (model ZX, Alfanuclear, Argentina, Buenos Aires) under optimal electronic conditions. All the animals were measured every day for 10 d. To determine zinc absorption, the ^{65}Zn radioactivity retained by each rat was measured by using a whole-body geometry: the animal was placed in a covered lucite box, and the size modified to the animal's size and to the detector geometry. In this way, it was possible to minimize detection errors during the measurements, which could be attributed to eventual movements of the animal. We determined the zinc-retention percentage for each rat as a function of time (Fig. 1). The zinc-absorption value was determined by extrapolating the final portion of the curve to the initial time ($t = 0$) by linear regression analysis of the experimental data to correct the data for physical radioisotopic decay and physiologic elimination.

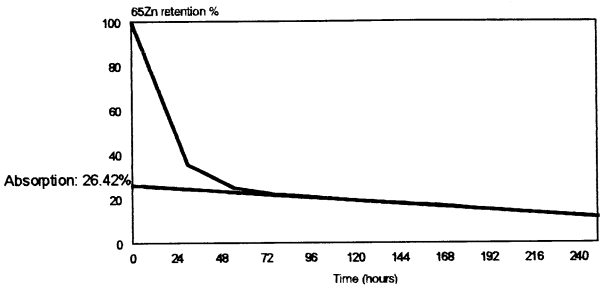

FIG. 1. Retention percentage of ^{65}Zn as a function of time in a male rat in the ^{65}Zn-gluconate group. The zinc absorption value for each rat was determined by the extrapolation of the final portion of the curve to the initial time ($t = 0$) by linear regression analysis of the experimental data to correct for the physical radioisotopic decay and physiologic losses.

Biodistribution Studies

For the biological distribution studies, animals were killed 10 d after the administration of the products. Rats were anesthetized with ethyl ether and bled by means of retroorbital sinus puncture to collect about 8 mL of blood from each rat. Liver, spleen, kidneys, stomach, duodenum, small intestine, gross intestine, pancreas, lungs, heart, muscle (quadriceps), bone (femur), and brain were removed from all animals, and testicles were removed from the males and the ovaries, uterus, and mammary glands were removed from the females. The organs were washed with isotonic saline solution, weighed, and measured in a gamma counter. The results were given as the percentage of radioactivity concentration (C%) and $C = A/w$, where A is the measured radioactivity and w is the weight of the organ.

Toxicity Studies

We used 50 male and 50 female 2-mo-old Sprague-Dawley rats that were assigned to groups of 10 animals to perform this study. Doses assayed were 1600, 1800, 2000, 2200, and 2400 mg of BioZn-AAS per kilogram of body weight.

Statistical Analysis

The data are presented as mean ± SD. The results were evaluated by a one-way analysis of variance. To test the differences among the means, we used the Student–Newman–Keuls method; $P < 0.05$ was considered statistically significant. The acute toxicity results were given as lethal dose 50 (LD_{50}), with limits according to the method proposed by Litchfield and Wilcoxon.[14]

RESULTS

Absorption Studies

Table I summarizes the absorption percentages of BioZn-AAS and the other sources used for comparative purposes for both sexes. Only in males did BioZn-AAS have a higher bioavailability ($P < 0.05$).

TABLE I.

ZINC ABSORPTION PERCENTAGES* FROM THE DIFFERENT ZINC SOURCES IN MALE AND FEMALE RATS

	% Absorption	
Zinc source†	Female	Male
^{65}Zn-sulphate	28.24 ± 4.60	23.15 ± 2.90
^{65}Zn-hydroxide	24.91 ± 4.02	22.62 ± 3.90
^{65}Zn-gluconate	25.51 ± 2.70	22.30 ± 3.90
^{65}Zn-BioZn-AAS	25.65 ± 2.20	27.97 ± 4.20‡

* Results are presented as mean ± SD. Absorption percentage values were obtained by extrapolating the final portion of the curve of retention percentage as a function of time for each rat by linear regression analysis.

† ^{65}Zn-sulphate, 10 female and 10 male rats; ^{65}Zn-hydroxide, 10 female and 10 male rats; ^{65}Zn-gluconate, 10 female and 10 male rats; ^{65}Zn-BioZn-AAS, 10 female and 10 male rats.

‡ Absorption percentage for BioZn-AAS in male rats was higher than that of the other sources ($P < 0.05$).

Biodistribution Studies

Tables II and III show the results of the biological distributions for male and female rats, respectively, 10 d after the administration of the products, to evaluate the metabolic pathway of each source assayed. There were significant differences in the C% values of the same organs with regard to the different zinc sources, but we considered that these differences were not physiologically relevant. Bone had the highest C% for both sexes. In the case of testicles for males and of liver, pancreas, and stomach for both sexes, we also found significant C% values.

Toxicity Studies

Table IV shows the results of the acute oral toxicity for BioZn-AAS. The value of LD_{50} for the female rats was 2000 mg/kg, with a lower limit of 1810 mg/kg and an upper limit of 2210 mg/kg. In the case of male rats, the LD_{50} value was 1900 mg/kg, with a lower limit of 1756 mg/kg and an upper limit of 2055 mg/kg.

DISCUSSION

Food fortification with an adequate zinc compound is an economic strategy to prevent zinc deficiency.[11,15–17] Until the present time, the major problem was finding a zinc compound that was adequate as a fortifying agent. Zinc sulfate and zinc oxide are the compounds used most often for food fortification, but they have serious disadvantages. Zinc sulfate modifies food sensorial characteristics, rendering the flavor unpalatable. Zinc oxide is insoluble and precipitates in liquid foods. In the case of solid foods, differences in granulometry and density between the particles of zinc oxide and those of the solid food ensure that zinc oxide remains at the bottom of the package, so that it does not interact with the nutritional matrix and is not available for consumption. These are the reasons these compounds are used only in low quantities and in solid foods. BioZn-AAS has the following technologic advantages: it has high solubility and a soft taste and it does not modify the sensorial characteristics of food.

Bioavailability studies showed that absorption of BioZn-AAS in aqueous solution did not differ from that of the other sources assayed, including zinc sulfate, the reference standard. Only in the case of males did BioZn-AAS have a higher bioavailability. However, because the experiment was performed with aqueous solutions, it would be interesting to study the absorption of each zinc compound in different nutritional matrixes. Such a study would be important because zinc is a hydrolytic metal that can form hydroxy metal polymers that precipitate in the intestine, thereby significantly affecting its absorption.[18] In the case of BioZn-AAS, gluconic acid and glycine, which are part of its composition, are

TABLE II.

BIOLOGICAL DISTRIBUTION RESULTS* FOR THE DIFFERENT ZINC SOURCES IN FEMALE RATS

Organ	^{65}Zn-Sulphate	^{65}Zn-Hydroxide	^{65}Zn-Gluconate	^{65}Zn-BioZn-AAS
Blood	2.22 ± 0.22	2.02 ± 0.17	2.21 ± 0.36	1.99 ± 0.21
Liver	6.92 ± 0.65	7.31 ± 0.84	7.13 ± 0.66	6.64 ± 1.86
Ovaries	3.21 ± 1.60	3.11 ± 1.58	3.96 ± 2.29	4.74 ± 2.08
Spleen	5.90 ± 1.56	5.05 ± 0.92	5.10 ± 0.68	5.54 ± 0.79
Kidneys	5.88 ± 0.33	5.81 ± 0.54	5.30 ± 0.34†	6.23 ± 0.88†
Stomach	7.08 ± 0.91	7.15 ± 0.44	6.92 ± 1.08	7.59 ± 0.63
Duodenum	5.06 ± 1.06	5.08 ± 0.87	4.86 ± 0.78	5.46 ± 1.48
Small intestine	3.69 ± 0.49	3.32 ± 0.67	3.71 ± 0.55	4.24 ± 0.97
Gross intestine	2.75 ± 0.43	2.74 ± 0.45	2.81 ± 0.38	2.83 ± 0.34
Pancreas	5.83 ± 1.51	6.33 ± 1.53	6.42 ± 0.82	6.84 ± 1.11
Lungs	4.78 ± 0.59	5.31 ± 1.32	4.98 ± 0.61	4.42 ± 0.44
Heart	4.88 ± 0.71	4.60 ± 0.76	4.65 ± 1.00	4.34 ± 0.64
Muscle	4.37 ± 0.21‡	4.19 ± 0.62	4.22 ± 0.32	3.84 ± 0.47‡
Bone	27.87 ± 3.45	28.18 ± 3.15	27.32 ± 2.39	25.12 ± 5.23
Brain	4.99 ± 0.75	4.79 ± 0.30	4.75 ± 0.61	4.71 ± 0.49
Mammary gland	1.90 ± 1.59	1.58 ± 0.72	1.50 ± 0.50	1.75 ± 0.51
Uterus	3.66 ± 1.87	3.24 ± 1.04	4.08 ± 2.42	3.64 ± 1.16

* Results are expressed as the percentage of radioactive concentration (%C) and presented as mean ± SD. Animals were killed 10 d after administration of the products.

† Kidneys: %C of ^{65}Zn-gluconate is lower than that of BioZn-AAS ($P < 0.05$).

‡ Muscle: %C of ^{65}Zn-sulphate is lower than that of BioZn-AAS ($P < 0.05$).

TABLE III.

BIOLOGICAL DISTRIBUTION RESULTS* FOR THE DIFFERENT ZINC SOURCES IN MALE RATS

Organ	^{65}Zn-Sulphate	^{65}Zn-Hydroxide	^{65}Zn-Gluconate	^{65}Zn-BioZn-AAS
Blood	2.22 ± 0.52	2.07 ± 0.33	2.14 ± 0.32	2.01 ± 0.21
Liver	7.61 ± 1.00	7.68 ± 1.08	7.44 ± 0.75	7.34 ± 0.53
Testicles	9.46 ± 1.61	9.20 ± 0.83	9.63 ± 0.70	9.63 ± 1.22
Spleen	5.24 ± 0.56	5.11 ± 0.77	5.23 ± 0.91	5.21 ± 1.05
Kidneys	5.77 ± 0.59	5.63 ± 0.49	6.07 ± 0.54	5.90 ± 0.26
Stomach	6.81 ± 0.85	6.78 ± 0.89	6.81 ± 0.59	8.01 ± 1.01†
Duodenum	5.20 ± 0.75	5.73 ± 1.16	4.48 ± 0.89	4.80 ± 0.69
Small intestine	3.74 ± 0.64	3.98 ± 0.70	3.57 ± 0.67	4.14 ± 0.41
Gross intestine	2.85 ± 0.16	3.15 ± 0.54	2.92 ± 0.44	2.80 ± 0.66
Pancreas	6.72 ± 1.06	7.95 ± 1.30‡	7.62 ± 2.05	6.01 ± 0.93‡
Lungs	4.29 ± 0.62	4.12 ± 0.40	4.56 ± 0.49	4.61 ± 0.45
Heart	5.22 ± 0.84	4.54 ± 0.59	4.72 ± 0.64	4.62 ± 0.56
Muscle	3.78 ± 0.74	3.65 ± 0.39	3.32 ± 0.65	3.66 ± 0.40
Bone	29.35 ± 5.63	25.93 ± 3.98	26.11 ± 4.80	26.87 ± 2.47
Brain	4.88 ± 0.66	4.32 ± 0.75	5.51 ± 1.92	4.60 ± 0.59

* Results are expressed as the percentage of radioactive concentration (%C) and presented as mean ± SD. Animals were killed 10 d after administration of the products.

† Stomach: %C of BioZn-AAS was higher than that of the other compounds ($P < 0.05$).

‡ Pancreas: %C of BioZn-AAS was different from that of ^{65}Zn-hydroxide ($P < 0.05$).

compounds that are considered "weak" ligands and that may compete with hydroxy polymerization and inhibit zinc precipitation. Thus, gluconic acid and glycine increase zinc absorption as many ligands from endogenous secretions do.[18] BioZn-AAS has two additional important advantages: its soft taste and high solubility. These properties may be due to the macromoleculelike structure of the compound, which prevents zinc from interacting with the nutritional matrix and thus modifying the sensorial characteristics of food.

Another important property of any compound used for food fortification is that it follow the same metabolic pathway as the the natural sources. Biological distribution studies showed that the metabolic behavior of BioZn-AAS does not differ from the other compounds assayed. Bone had the highest zinc concentration, in agreement with the bibliographic data for growing animals.[19–21] Zinc is essential for bone mineralization, and it is bound to the mineral matrix, where this process starts.[21–23] Several studies have demonstrated that zinc deficiency during pregnancy results in severe malformations of the fetus, especially in skeletal growth and calcification.[21,23] In our study, the activity concentration found in bone was localized in the mineral matrix, as demonstrated by the negligible activity concentration in the bone marrow after it was separated from the mineral matrix (data not shown). There is controversy about the role of skeletal zinc as an available reservoir. In those cases in which bone remodeling is an active process, as in growing animals, zinc may be mobilized for metabolic use.[19] This would not be the case for adult animals because bone zinc appears not to be mobilized for metabolic functions or to supply the needs of normal fetal development of zinc-deficient pregnant rats.[20,23] Zinc concentration in skeleton in adult animals is lower than that in younger animals, and bone remodeling also is lower in adults. In a preliminary study performed at our laboratory with 6-mo-old adult male and female Sprague-Dawley rats weighing 350–420 g, we found bone C% values of 10.95% ± 2.47% for females and 9.5% ± 3.82% for males. These results, which are significantly different from those shown in Tables II and III, respectively, indicate that the bone zinc pool is not so important in adult animals but is essential for those animals under active growth and development. It is important that the diet provides an adequate amount of zinc, especially for children, to prevent or correct growth retardation.[16,24] Despite the lower values of zinc concentration in organs other than bone, zinc metalloenzymes are widespread throughout body tissues and play crucial roles in many physiologic processes.[5] Other organs that had significant C% values were the liver, pancreas, and testicles; the liver and pancreas are closely associated with zinc metabolism,[5] and zinc is an important cofactor for several processes such as spermatogenesis and steroidgenesis in the testicles.[25]

Another important property for a compound used for food fortification is its toxicity because of the concentration used in fortifying the food. The LD_{50} for BioZn-AAS was similar to those of the other sources. These experimental results may be explained by the macromoleculelike structure of BioZn-AAS, which probably prevents its deleterious effect on the gastrointestinal mucosa.

In conclusion, the present results show that BioZn-AAS is a promising source of zinc for food fortification or dietary supplementation to prevent zinc deficiency. This compound has the same bioavailability as the reference standard (zinc sulfate), with the same metabolic behavior, and toxicity values similar to those of the other assayed sources. BioZn-AAS has several other important characteristics with regard to food fortification: its high solubility,

TABLE IV.

ACUTE ORAL TOXICITY OF BioZn-AAS IN RATS: VALUES OF LD_{50} AND ITS CONFIDENCE LIMITS

Sex	*n* animals	LD_{50}*	Lower limit*	Upper limit*
Female	50	1900	1756	2055
Male	50	2000	1810	2210

* Presented as milligrams of BioZn-AAS per kilogram of body weight.

soft taste, low cost and the fact that it does not modify the sensorial characteristics of food. Additional studies are needed to confirm its potential use in improving human nutrition.

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