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

María J. Salgueiro, Bioch, Marcela B. Zubillaga, PhD, Alexis E. Lysioneck, Pharm, María I. Sarabia, Pharm, Ricardo A. Caro, PhD, Tomás De Paoli, PhD, Alfredo Hager, PhD, Eduardo Ettlin, Pharm, Ricardo Weill, Eng, and José R. Boccio, Bioch

From the Radioisotope Laboratory and the Physics Department, School of Pharmacy and Biochemistry, University of Buenos Aires; and the Agrarian Industries Department, School of Agronomy, University of Morón, Buenos Aires, Argentina

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 $\mu$g/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% ± 2.20% for BioZn-AAS, 28.24% ± 4.60% for ZnSO$_4$, 24.91% ± 4.02% for Zn(OH)$_2$, and 25.51% ± 2.70% for Zn-gluconate). In the case of the male rats, absorption of BioZn-AAS (27.97% ± 4.20%) was higher ($P<0.05$) than that from the other compounds (23.15% ± 2.90% for ZnSO$_4$, 22.62% ± 3.90% for Zn(OH)$_2$, and 22.30% ± 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

Key words: zinc, bioavailability, metabolism, toxicity, rats

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 zinc3 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.9,9

Oral acute toxicity data for humans, rats, and mice10 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-
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. This dose was administered in water solution at a final volume of 1 mL per rat. All compounds were intrinsically labeled with 65Zn except ZnSO₄, which was labeled by isotopic exchange by leaving the preparation overnight. The activity administered to each animal was 0.33 MBq of 65Zn (NEN; Du Pont, Buchs, Switzerland; catalog no. NEZ-109, Wilmington, Delaware, USA), which was in the chemical form of ZnCl₂ in 0.5 mol/L of HCl (specific activity = 3.83 GBq/mg, activity concentration = 0.25 GBq/mL), was used for labeling.

**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. This dose was administered in water solution at a final volume of 1 mL per rat. All compounds were intrinsically labeled with 65Zn except ZnSO₄, which was labeled by isotopic exchange by leaving the preparation overnight. The activity administered to each animal was 0.33 MBq of 65Zn (NEN; Du Pont, catalog no. NEZ-109, Wilmington, Delaware, USA), which was in the chemical form of ZnCl₂ in 0.5 mol/L of HCl (specific activity = 3.83 GBq/mg, activity concentration = 0.25 GBq/mL), was used for labeling.

65Zn-SULFATE. We labeled 0.425 mg of ZnSO₄ · 7H₂O (Fluka, Buchs, Switzerland; catalog no. 96501) by an isotopic exchange with 6.66 MBq of 65Zn and completed the preparation to a final volume of 20 mL with deionized water.

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

65Zn-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 65Zn. 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 65Zn-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 (TI) 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 65Zn 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 for the physical radioisotopic decay and physiologic elimination.

**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 (LD₅₀), 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).
\section*{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.

\section*{Toxicity Studies}

Table IV shows the results of the acute oral toxicity for BioZn-AAS. The value of LD\textsubscript{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\textsubscript{50} value was 1900 mg/kg, with a lower limit of 1756 mg/kg and an upper limit of 2055 mg/kg.

\section*{DISCUSSION}

Food fortification with an adequate zinc compound is an economic strategy to prevent zinc deficiency. 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 matrices. 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. In the case of BioZn-AAS, gluconic acid and glycine, which are part of its composition, are

\begin{table}[h]
\centering
\caption{Zinc absorption percentages* from the different zinc sources in male and female rats}
\begin{tabular}{lcc}
\hline
Zinc source† & Female & Male \\
\hline
\textsuperscript{65}Zn-sulphate & 28.24 ± 4.60 & 23.15 ± 2.90 \\
\textsuperscript{65}Zn-hydroxide & 24.91 ± 4.02 & 22.62 ± 3.90 \\
\textsuperscript{65}Zn-gluconate & 25.51 ± 2.70 & 22.30 ± 3.90 \\
\textsuperscript{65}Zn-BioZn-AAS & 25.65 ± 2.20 & 27.97 ± 4.20‡ \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Biological distribution results* for the different zinc sources in female rats}
\begin{tabular}{lcccc}
\hline
Organ & \textsuperscript{65}Zn-Sulphate & \textsuperscript{65}Zn-Hydroxide & \textsuperscript{65}Zn-Gluconate & \textsuperscript{65}Zn-BioZn-AAS \\
\hline
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 \\
\hline
\end{tabular}
\end{table}
TABLE III.

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

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

* 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 65Zn-hydroxide (P < 0.05).

TABLE IV.

ACUTE ORAL TOXICITY OF BIOZn-AAS IN RATS: VALUES OF LD50 AND ITS CONFIDENCE LIMITS

<table>
<thead>
<tr>
<th>Sex</th>
<th>n animals</th>
<th>LD50*</th>
<th>Lower limit*</th>
<th>Upper limit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>50</td>
<td>1900</td>
<td>1756</td>
<td>2055</td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>2000</td>
<td>1810</td>
<td>2210</td>
</tr>
</tbody>
</table>

* 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.

REFERENCES


6. Evans GW, Johnson EC. Effect of iron, vitamin B-6 and picolinic acid on zinc absorption in the rat. J Nutr 1981;111:68


10. RTECS. Database compiled by the National Institute of Occupational Safety and Health, U. S. Department of Health and Human Services, 1997


25. Aeson O, Chung KW. Dietary zinc deficiency alters 5α-reduction and aromatization of testosterone and androgen receptors in rat liver. J Nutr 1996;126:842