Influence of zinc on the status of hepatic trace elements and biokinetics of $^{65}$Zn in ethanol treated rats

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Whole body counting studies of $^{65}$Zn indicated that the T$_{1/2}$ (the faster component) was significantly decreased while the slower component (T$_{9/2}$) was increased significantly following ethanol treatment. Interestingly, following zinc treatment to ethanol treated rats, slower component (T$_{9/2}$) of $^{65}$Zn came back to within normal limits while the faster component (T$_{1/2}$) got significantly elevated in comparison to ethanol treatment. Percent uptake values of $^{65}$Zn were found to be increased in liver, intestine, muscle, brain and kidney, and decreased in bone under alcoholic conditions. Interestingly, the uptake values of $^{65}$Zn in all the organs except muscle were reverted back to within normal limits upon zinc supplementation to these ethanol intoxicated animals. A significant decrease in zinc contents was noticed in ethanol treated rats, which, however, were raised to normal levels upon zinc supplementation. Copper levels, on the other hand, were significantly enhanced in both ethanol fed and combined ethanol + zinc treated rats. Calcium levels were significantly decreased in both ethanol and zinc treated rats, which however were further reduced upon zinc supplementation to ethanol fed rats. However, no significant change was observed in the concentrations of sodium and potassium in any of the treatment groups. In conclusion, zinc appears to play a protective role by normalizing the turnover of $^{65}$Zn in whole body as well as in its uptake in different organs under alcoholic conditions.

Keywords: $^{65}$Zn biokinetics, Hepatic trace elements, Ethanol

Excessive consumption of ethanol is recognized as among the most prevalent known causes of abnormal human development. Chronic and excessive use of alcohol leads to bizarre physiological changes including tolerance or resistance, addiction, organ damage and a whole range of biochemical and neurological lesions. Abnormalities in zinc metabolism are common in patients with chronic alcoholism. Zinc, as a trace element, is regarded as an essential nutrient for human beings and has also been found to be protective in some kind of liver injury. Alcoholic cirrhosis may be associated with a state of zinc deficiency. It is known that ethanol exposure leads to zinc mobilization from various tissues. The tissue deposition and mobilization of zinc are affected by a number of pathophysiological processes. The information about the biological half-life of $^{65}$Zn could be useful for understanding the homeostatic mechanisms of zinc in the body. The radiotracer using $^{65}$Zn indicated variations in its uptake by different tissues with liver storing the maximum amount.

Metabolism and toxicity of metals may be influenced by ethanol and its metabolites, which are capable of producing secondary deficiency by interfering with the metabolism of essential nutrients and trace metals through effects on their absorption, redistribution of excretion. Zinc is known to compete with cadmium, lead, copper, iron and calcium for similar binding sites. In alcoholism, serum concentrations of zinc were found to be diminished whereas levels of copper were either increased or normal. The reduced levels of these trace elements may have other clinical implications in conditions of alcoholism.

Therefore, in view of the effects of ethanol intake on the metabolism of zinc and its interaction with other trace metals under alcoholic conditions, the present study has been designed to examine the effects of zinc alone and in combination with ethanol on the biokinetics of $^{65}$Zn in the whole body and its relationship with other elements.

Materials and Methods

Animal groups—Male wistar rats weighing 100-120g were divided into four main groups of 6-8 animals each. Animals in group I (normal control)
were fed normal pelleted diet and water ad libitum. Rats in group 2 (ethanol treated) were fed daily with 3ml of 30% ethanol orally. Rats belonging to group 3 (zinc treated) were supplemented with zinc in the form of zinc sulphate (ZnSO₄,7H₂O) at a dose level of 227mg/l mixed in their drinking water. Rats in group 4 (zinc + ethanol treated) were given 3 ml of 30% ethanol as given in group 2 and zinc as in group 3 animals. All the treatment in various groups were continued for a total duration of 8 weeks.

Radioisotopic studies—Whole body biological half-life of ⁶⁵Zn: Each rat was injected with 1.85 MBq of ⁶⁵Zn (5.11mCi/g) after 8 weeks of different treatments and the radioactivity was recorded by placing the rat in a Perspex cage (18 x 8 x 6 cm) at a distance of 17cm from the surface of the probe of gamma ray spectrometer (ECIL, Hyderabad, India). At this distance, the variation in the count rate due to the sideways movement of the animal, if any, was negligible. During the course of recording radioactivity, five sets of measurements counts were taken on each animal in order to minimize the statistical error. The standard activity of ⁶⁵Zn (equivalent to that injected to each animal) was also measured to account for the physical decay of the radioisotope and the possible instrument error. All the treatments were continued until the uptake studies were completed. The experiment continued for 15 days. To determine the whole body biological half-life (Tb, biol), the percentage uptake values of ⁶⁵Zn at different time intervals from day 1 onwards were calculated by taking the day 1 uptake as 100%. The percent whole body uptake values were plotted (y-axis - log scale) as a function of time (x-axis - linear scale) on the semi log paper. Further, the Tb, biol and Tb, 2 of ⁶⁵Zn were interpolated from the semi-log plot.

Distribution of ⁶⁵Zn in different organs—All the rats from different groups were injected with a tracer dose of 0.925 MBq ⁶⁵Zn and were sacrificed 24hr after injection by exsanguination under light ether anaesthesia. The organs viz: brain, liver, kidney, intestine, muscle and bone were taken out and the wet weight of all the organs were recorded. Each organ was placed in a test tube containing 3ml of 30% KOH for digestion. On the next day, the activity of the digested fraction of the tissue was noted using NaI (Tl) scintillation counter (ECIL, Hyderabad, India). The percentage uptake in each organ with same weight was calculated with respect to a standard having the same activity as that injected.

Trace elemental analysis—After 8 weeks of various treatments, overnight fasted animals were sacrificed under light ether anaesthesia. Livers were removed, weighed and digested in 1:4 mixture of concentrated perchloric acid and nitric acid and heated till the acid mixture evaporated fully. The residue of all the samples was reconstituted in 5ml of 10mM nitric acid and analyzed using Perkin Elmer Atomic Absorption Spectrophotometer-3100. The levels of sodium, potassium and calcium were estimated using Systromic type-121 flame photometer.

Statistical analysis—The statistical significance of the data has been made using one-way analysis of variance (ANOVA) followed by Newman Keuls test as mean ± SD.

Results
Whole body studies indicated that the faster component of the whole body biological half-life of ⁶⁵Zn (Tb, biol) was significantly decreased following ethanol feeding to normal rats. It also got decreased significantly when the rats were given combined zinc and ethanol treatment as compared to the normal controls. On the contrary, the slower component of the whole body biological half-life of ⁶⁵Zn i.e. Tb, 2, was increased significantly following ethanol treatment when compared to the normal control. However, it was not affected upon zinc treatment. Interestingly, Tb, 2 of ⁶⁵Zn, which got elevated upon zinc treatment, was normalized when zinc was supplemented to ethanol treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tb, biol</th>
<th>Tb, 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>5.27 ± 0.66</td>
<td>14.80 ± 3.06</td>
</tr>
<tr>
<td>II Ethanol</td>
<td>3.31 ± 0.18</td>
<td>17.50 ± 1.68</td>
</tr>
<tr>
<td>III Zinc</td>
<td>6.05 ± 1.39</td>
<td>12.68 ± 1.57</td>
</tr>
<tr>
<td>IV Zinc + ethanol</td>
<td>4.52 ± 0.15</td>
<td>13.44 ± 2.02</td>
</tr>
</tbody>
</table>

P values: *<0.05, **<0.01, ***<0.001 by Newman-Keuls test when the values of group II, III and IV are compared with those of group I; *<0.01 by Newman-Keuls test when the values of group IV are compared with those of group III; *<0.05, **<0.01 by Newman-Keuls test when the values of group IV are compared with those of group II.
A statistically significant increase was noticed in percent uptake values of \(^{65}\)Zn in liver, intestine, brain and muscle, whereas a significant decrease was observed in bone following ethanol feeding, when compared to the normal control. Interestingly, the uptake values in all the organs were normalized except in muscle when zinc was supplemented to ethanol fed rats. Further, a significant decrease in the percent uptake of \(^{65}\)Zn was noticed in liver, intestine and brain on combined treatment group, when the results were compared with the ethanol fed ones (Table 2).

The concentrations of zinc in liver was found to be decreased significantly following ethanol treatment whereas it got increased significantly following \(\text{per se}\) zinc treatment to normal rats and ethanol fed rats (Table 3). On the other hand, the copper levels were found to be enhanced significantly following ethanol feeding as well as upon combined zinc and ethanol treatments, as compared to normal controls. However, no significant change was observed in the copper levels in zinc treated animals. Further, no significant change was observed in the concentrations of sodium and potassium in animals belonging to all the treatment groups. Interestingly, the levels of calcium were found to be significantly decreased in animals of all the treatment groups.

**Discussion**

The decrease in the \(T_{b1}\) component of \(^{65}\)Zn from the whole body, as observed in the present study, would correspond to its increased requirement, which possibly could be due to its faster elimination in alcoholic conditions. Ethanol treatment has been associated with low levels of zinc, which could be the result of its faster elimination in alcoholic condition and also be the result of displacement of zinc from zinc binding ligands. The decrease in biological half-life upon combined zinc and ethanol treatment could also be due to non-availability or saturation of binding sites for \(^{65}\)Zn due to competition between zinc and \(^{65}\)Zn for similar binding sites. Increase in dietary zinc intake led to increase in metallothionein levels in liver\(^{16}\). However, to combat the decreased concentration of zinc in alcoholic conditions, the body seemed to have adapted to the decreased turnover of \(^{65}\)Zn as indicated by increased \(T_{b2}\) which could possibly be due to decrease in the mobilization of zinc.

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**Table 2**—Distribution of \(^{65}\)Zn in different organs following zinc treatment to ethanol fed rats (Percent uptake of administered dose)

[Values are expressed as mean ± SD of 6-8 animals]

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Intestine</th>
<th>Muscle</th>
<th>Bone</th>
<th>Brain</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>1.65 ± 0.28</td>
<td>1.08 ± 0.40</td>
<td>0.27 ± 0.03</td>
<td>0.86 ± 0.17</td>
<td>0.14 ± 0.02</td>
<td>0.79 ± 0.18</td>
</tr>
<tr>
<td>II Ethanol</td>
<td>2.31 ± 0.20</td>
<td>1.58 ± 0.25</td>
<td>0.42 ± 0.07</td>
<td>0.68 ± 0.05</td>
<td>0.19 ± 0.03</td>
<td>0.88 ± 0.05</td>
</tr>
<tr>
<td>III Zinc</td>
<td>1.77 ± 0.45</td>
<td>1.46 ± 0.42</td>
<td>0.26 ± 0.09</td>
<td>0.51 ± 0.08</td>
<td>0.16 ± 0.04</td>
<td>0.84 ± 0.05</td>
</tr>
<tr>
<td>IV Zinc + ethanol</td>
<td>1.75 ± 0.08</td>
<td>0.88 ± 0.16</td>
<td>0.88 ± 0.16</td>
<td>0.72 ± 0.10</td>
<td>0.14 ± 0.01</td>
<td>0.89 ± 0.04</td>
</tr>
<tr>
<td>F-value</td>
<td>5.65</td>
<td>7.641</td>
<td>10.889</td>
<td>8.455</td>
<td>3.399</td>
<td>1.125</td>
</tr>
<tr>
<td>P-values</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 3**—Effect of zinc on the elemental concentrations in liver samples of ethanol treated rats.

[Values are expressed as mean ± SD of 6-8 animals]

<table>
<thead>
<tr>
<th>Group</th>
<th>Zinc (µg/g)</th>
<th>Copper(µg/g)</th>
<th>Sodium(mg/g)</th>
<th>Potassium(mg/g)</th>
<th>Calcium(µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>110.8 ± 8.15</td>
<td>30.28 ± 7.91</td>
<td>90.81 ± 9.01</td>
<td>6.68 ± 0.79</td>
<td>427.4 ± 33.58</td>
</tr>
<tr>
<td>II Ethanol</td>
<td>78.28 ± 6.72</td>
<td>46.69 ± 4.00</td>
<td>99.99 ± 10.45</td>
<td>6.78 ± 0.32</td>
<td>388.7 ± 13.56</td>
</tr>
<tr>
<td>III Zinc</td>
<td>160.2 ± 10.24</td>
<td>26.13 ± 4.32</td>
<td>93.50 ± 10.87</td>
<td>6.65 ± 0.77</td>
<td>352.9 ± 26.04</td>
</tr>
<tr>
<td>IV Zinc + ethanol</td>
<td>146.0 ± 10.10</td>
<td>42.75 ± 4.65</td>
<td>96.12 ± 7.32</td>
<td>6.97 ± 0.31</td>
<td>327.62 ± 17.56</td>
</tr>
<tr>
<td>F-value</td>
<td>6.633</td>
<td>29.975</td>
<td>1.242</td>
<td>1.075</td>
<td>18.014</td>
</tr>
<tr>
<td>P-values</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P values: \(a<0.05, b<0.01, c<0.001\) by Newman–Keuls test when the values of group II, III and IV are compared with those of group I; \(b<0.01\) by Newman–Keuls test when the values of group IV are compared with those of group III; \(c<0.05, d<0.01\) by Newman–Keuls test when the values of group IV are compared with those of group II.
metallothionein stores in zinc deficient conditions caused by ethanol intake.17,18

Liver is the organ showing the highest percent specific uptake in comparison to other organs. The high uptake of $^{65}$Zn in liver and its slices following ethanol treatment may be explained either on the basis of metallothionein induction in the liver, which has good binding affinity for zinc19 or decrease in the concentration of zinc in alcoholism which lead to more uptake in such conditions. The uptake studies also serve as an index of zinc absorption in the intestine in conditions of ethanol and zinc treatment. The high uptake of $^{65}$Zn in brain, could be to overcome the adverse effects of ethanol in neurotransmission and in kidney, rich vascularisation may be the reason for slightly increased $^{65}$Zn uptake following ethanol ingestion. There is a competitive binding of zinc and $^{65}$Zn at the same binding site and this accounts for $^{65}$Zn uptake being normalized upon zinc supplementation. This observation thus substantiates the protective effect of zinc supplementation in ethanol-intoxicated animals.

It is well recognized that liver plays an important role in the regulation of metabolism of trace elements.19 Imbalances in the supply of any of the essential elements in the body can have both nutritional and toxicological consequences with regard to the metabolism of other metals. They can further be responsible for the development of clinical signs of trace element deficiencies or can modify the susceptibilities to metal toxicities.20 The present study indicated that the concentration of zinc was decreased significantly following ethanol treatment.21 This could be due to low level of zinc and increased urinary losses of zinc. It has also been evidenced that as metallothionein induction is in response to zinc supplementation, significant reserves of zinc from the body are utilized in the synthesis of metallothionein in response to any toxicity, thus causing a net deficit of free circulating plasma zinc concentrations. Therefore, the increase in uptake of $^{65}$Zn in liver following ethanol treatment, which may be due to increased requirement of zinc in alcoholic conditions, also substantiates the decreased concentration of zinc in liver. Thus, some alteration in the transport or metabolism of zinc in toxic conditions afforded by ethanol leads to lowered zinc concentrations. Restoration of normal zinc levels upon its supplementation to ethanol fed rats confirms that body zinc content has a direct bearing on dietary zinc levels.

Zinc has been shown to interact with various metals like copper and cadmium, and is considered to be of clinical significance for humans and animals. Ethanol feeding results in lower levels of zinc and this, in turn, results in increased copper levels22 as observed in the present study, thus indicating their antagonistic nature towards each other. The antagonistic effect of copper towards zinc is likely due to the binding of copper with the target ligands in conditions of zinc deficiency. It has been evidenced, the interaction between copper and zinc is mediated through metallothionein23 and increase in copper levels may be related to increased metallothionein synthesis24 and also due to competition for available binding sites.25 The essential minerals, calcium and zinc, serve unique functions in higher organisms, and it is well recognized that homeostatic mechanisms are involved in regulating their metabolism. In the present study, the calcium concentrations were found to be decreased significantly after ethanol feeding. This could be due to the antagonistic effect of zinc on calcium.26 It has also been reported that Zn+ may suppress Ca++ effect by displacement of calcium ions from its cell binding sites27, thus altering the membrane calcium pump resulting in a reduction in free intracellular calcium. Further decline in calcium in combined zinc and ethanol treated rats may be due to the combined effect of both zinc and ethanol. However, no significant change was observed in the concentrations of sodium and potassium in any of the treatment groups, indicating that the status of these ions did not alter under alcoholic conditions or following zinc supplementation.

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References


