Vitamin B Complex in Treatment of Cadmium Intoxication

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ABSTRACT

The effect of vitamin B-complex on cadmium nephrotoxicity and hepatotoxicity was investigated in rats. The administration of Cd (3 mg per kg, s.c., three days) increased the urinary excretions of lactic dehydrogenase (LDH), glutamic oxalacetic transaminase (GOT), and total proteins, decreased renal activities of LDH and GOT and increased concentration in kidney tissue of Cd, Cu, and Zn. Cadmium also increased serum GOT and glutamic pyruvic transaminase (GPT), decreased hepatic activities of GOT and GPT, and increased hepatic levels of Cd and Zn. The supplementation of vitamin B-complex (10 mg per kg, orally) simultaneously with Cd caused less marked biological alterations. Cadmium concentration in renal tissue was significantly less on the eighth day whereas the hepatic level of Cd was unaffected by vitamin supplementation. The protective effect of vitamin B-complex in Cd toxicity may be attributed to the interference by the constituents of vitamin B-complex in body absorption of Cd, possibly through forming readily excretable complexes. The results suggest that Cd toxicity can be reduced by vitamin B-complex supplementation.

Introduction

Cadmium is an occupational and environmental pollutant and causes liver, kidney, and pancreas dysfunction. Chelation therapy is most effective in heavy metal poisoning. However, some of the chelating agents may cause nephrotoxicity, hypocalcemia, hypotension, anemia, damage to intestinal mucosa, and essential metal imbalance. This has led to a search for non-toxic therapeutic agents for metal poisoning. Vitamins B₉, B₁₂, and folic acid have been reported to reduce the severity of lead poisoning, including decrease in the blood lead levels and basophilic stippling in rabbits. Thiamine (vitamin B₁) has been shown to prevent accumulation of lead in tissues and clinical signs of lead intoxication in calves. Industrial workers in some countries may suffer from vitamin deficiency and this may be associated with exposure to heavy metals. The dietary supplementation of vitamins may, therefore, be expected to ameliorate metal intoxication. It has been

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observed that simultaneous supplementation of vitamin B-complex prevented tissue accumulation of lead and reduced biochemical alterations in liver, kidney, and brain of rats with lead poisoning.6

The present communication deals with the effects of simultaneous treatment of vitamin B-complex on cadmium nephrotoxicity and hepatotoxicity. The hepatic injury was evaluated by determining the activities of diagnostic enzymes in serum and liver and the renal injury by measuring the urinary excretion of enzymes, total proteins and the activities of enzymes in kidney tissue. The hepatic and renal levels of Cd, Cu, and Zn were also measured to study the influence of vitamin B-complex on the uptake and retention of Cd in relation to trace metals.

Materials and Methods

Thirty male albino rats of ITRC colony (body weight 150 ± 10 g) maintained on standard pellet diet* and water ad libitum were divided equally into three groups. The animals of group I were administered 3 mg per kg, Cd as CdCl₂, H₂O subcutaneously, dissolved in normal saline (2 ml per kg) and those of group II were administered 3 mg per kg, Cd subcutaneously plus 10 mg per kg, vitamin B-complex (Complex B Forte tablet†) dispensed in normal saline (2 ml per kg) orally, once daily for three days. The animals of group III received an equal volume of normal saline and served as controls. During the treatment, the animals of each group were housed separately in metabolic cages (two per cage) and 24 hr urine was collected in cold tubes, consecutively for three days.

Urine samples were centrifuged (3,000 g for 10 min) and the supernatant dialysed against ice cold water for three hrs to remove enzyme inhibitors. Half of the animals in each group were killed by decapitation on the fourth day and the remaining half on the eighth day. Liver and kidneys were removed, washed free of extraneous material, weighed and homogenized by six strokes of a motor driven teflon-glass tissue homogenizer in ice cold 0.25 M sucrose (10 percent w/v). Blood was obtained by cardiac puncture.

Enzyme Assays

Enzyme assays were done at the predetermined respective pH optima and the activities were in the proportionality range with respect to amount of enzyme and the incubation period.

\textit{Glutamic oxalacetic transaminase (GOT, E.C. 2.6.1.1.)} \textit{Glutamic pyruvic transaminase (GPT, E.C. 2.6.1.2.)} The activity of GOT in urine, serum, liver, and kidneys and the activity of GPT in serum and liver were measured according to the procedure of Reitman and Frankel14 by estimating their respective hydrazones at 510 nm.

\textit{Lactic dehydrogenase (LDH, E.C. 1.1.1.27)} The enzyme activity in urine and kidneys was measured as described by Wooton22 by following the rate of NADH oxidation at 340 nm.

Estimation of Total Protein

The urinary total protein was estimated by the procedure of Piscator.12 The protein was precipitated by Tsuichiya’s reagent (a mixture of phosphotungstic acid, hydrochloric acid, and ethanol) and reacted with Biuret reagent. The absorbance was read at 330 nm.

The method of Lowry et al8 was employed to estimate total protein in liver and kidneys. The protein was pre-

* Hindustan Lever Ltd., India.
† Composition of Complex B Forte tablet: Vitamin B₁₂ I.P. 10 μg, Vitamin B₁ I.P. 50 mg, Riboflavin I.P. 10 mg, Nicotinamide I.P. 70 mg, Calcium pentothenate USP 10 mg, Folic acid I.P. 5 mg and Vitamin B₆ I.P. 2.5 mg. (Glaxo Company, India).
VITAMIN B COMPLEX IN TREATMENT OF CADMIUM INTOXICATION

Figure 1. The effect of Cd or Cd + vitamin B-complex administration on the urinary excretion of LDH, GOT, and protein in rat (mean ± S.E. of five values; a_p < 0.001, b_p < 0.01, c_p < 0.05, when compared to control (mean ± S.E. of five values) shown by horizontal line, **_p < 0.01, *_p < 0.05 when compared to corresponding Cd group as evaluated by the student's "t" test.

capitated by 10 percent trichloroacetic acid followed by solubilization in dilute alkali (0.1N). The color was developed with the Folin reagent, and the absorbance read at 660 nm.

Estimation of Metals

Weighed samples of kidneys and liver were digested with an acid mixture consisting of HNO_3:HCl:H_2SO_4 (6:1:1). The resulting carbon free residue was dissolved in five percent HNO_3 and determinations of Cu, Zn, and Cd contents at 324.7, 213.8 and 228.8 nm, respectively were read on a flame atomic absorption spectrometer* using high intensity hollow cathode lamps. Standards for each metal were analysed simultaneously.

Results

Administration of Cd significantly increased the urinary excretion of LDH, GOT, and total proteins indicating nephrotoxicity of Cd. The simultaneous supplementation of vitamin B-complex with Cd significantly reduced these effects (figure 1). Cadmium decreased the activities of renal LDH and GOT as measured on the fourth day but returned to normal by the eighth day, indicating recovery from early renal damage (table I). The levels of renal Cd, Cu, and Zn increased significantly following four and eight days of Cd administration. The simultaneous

| TABLE I |
|-----------------|-----------------|-----------------|-----------------|
|                | Control         | Day 4           | Day 8           | Cd + Vitamin B-Complex Day 8 |
| LDH*           | 2.24 ± 0.08     | 1.95 ± 0.13     | 2.17 ± 0.13     | 3.44 ± 0.43§     |
|                 | (6)             | (5)             | (5)             | (5)              |
| GOT†           | 22.84 ± 1.80    | 17.14 ± 1.58§   | 20.76 ± 1.31    | 19.80 ± 2.32     |
|                 | (5)             | (5)             | (5)             | (5)              |
| Cd‡            | 0.08 ± 0.00     | 12.60 ± 0.84§   | 18.75 ± 2.20§   | 12.01 ± 0.71§    |
|                 | (5)             | (5)             | (5)             | (5)              |
| Cu‡            | 5.76 ± 0.93     | 7.33 ± 1.29§    | 6.08 ± 0.25     | 11.15 ± 2.27§    |
|                 | (5)             | (5)             | (5)             | (5)              |
| Zn‡            | 21.0 ± 1.30     | 28.60 ± 2.90§   | 28.86 ± 2.97§   | 29.00 ± 2.84§    |
|                 | (5)             | (5)             | (5)             | (5)              |

*µMole NADH oxidized per minute per mg of protein.
†µMole hydrazine formed per minute per mg of protein.
‡µg per g of fresh tissue.
Each figure is mean ± S.E. of number of rats given in parenthesis.
§_p < 0.001 compared to control.
* _p < 0.01 compared to control.
* _p < 0.05 compared to control.
* _p < 0.01 compared to corresponding Cd group as evaluated by Student's t test.
* _p < 0.05 compared to corresponding Cd group as evaluated by Student's t test.

* Perkin Elmer, model 5,000.
supplementation of vitamin B-complex with Cd reduced the inhibition in the activities of these enzymes. The enhanced level of renal Cd, however, remained unaffected by simultaneous treatment with vitamin B-complex on the fourth day but reduced significantly on the eighth day (table I). The renal concentration of Cu increased while that of Zn remained elevated on the fourth day in vitamin B-complex supplemented rats. The levels of these metals returned to normal on the eighth day.

The activities of GOT and GPT increased in serum (figure 2) and decreased in the liver (table II) on exposure to Cd and reflects liver damage. The concentration of Cd and Zn also increased in the liver (table II) whereas the hepatic Cu content remained unaltered. The concomitant administration of vitamin B-complex with Cd reduced these enzyme alterations, while the hepatic levels of Cd and Zn remained elevated.

**Discussion**

Enzymuria, proteinuria, elevation in serum enzymes, and inhibition in the activities of renal and hepatic enzymes are manifestations of early Cd hepatorenal toxicity.\(^2,9,23\) Since Cd is a known inducer of metallothionein, the uptake of Cu and Zn in the renal tissue also increased significantly with the renal concentration of Cd.\(^10\) The increased uptake of hepatic Zn in animals admin-

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### TABLE II

**Effect of Cadmium and Cadmium Plus Vitamin B-Complex Treatment on Rat Hepatic Enzymes and Metal Contents**

<table>
<thead>
<tr>
<th></th>
<th>Control 4</th>
<th>Cd 4</th>
<th>Cd 8</th>
<th>Cd + Vitamin B-Complex 4</th>
<th>Cd + Vitamin B-Complex 8</th>
</tr>
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<tbody>
<tr>
<td><strong>GOT</strong> *)</td>
<td>28.42 ± 1.78</td>
<td>22.61 ± 1.25*</td>
<td>17.24 ± 2.15§</td>
<td>21.53 ± 1.76*</td>
<td>24.98 ± 0.59*</td>
</tr>
<tr>
<td>(6)</td>
<td>(5)</td>
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<td>(5)</td>
<td>(5)</td>
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<tr>
<td><strong>GPT</strong> *)</td>
<td>27.11 ± 1.49</td>
<td>20.32 ± 2.51*</td>
<td>17.82 ± 2.35§</td>
<td>30.62 ± 5.98*</td>
<td>25.43 ± 2.73*</td>
</tr>
<tr>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
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<td>(5)</td>
</tr>
<tr>
<td><strong>Cd</strong> †</td>
<td>0.24 ± 0.11</td>
<td>14.27 ± 2.26‡</td>
<td>15.15 ± 1.08‡</td>
<td>13.77 ± 0.32‡</td>
<td>14.06 ± 1.02‡</td>
</tr>
<tr>
<td>(6)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
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</tr>
<tr>
<td><strong>Cu</strong> †</td>
<td>3.51 ± 0.71</td>
<td>2.95 ± 0.56</td>
<td>3.01 ± 0.67</td>
<td>2.87 ± 0.23</td>
<td>3.32 ± 0.23</td>
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<td>(5)</td>
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<tr>
<td><strong>Zn</strong> †</td>
<td>46.84 ± 1.00</td>
<td>51.74 ± 0.79*</td>
<td>55.00 ± 3.79*</td>
<td>57.03 ± 3.23*</td>
<td>58.42 ± 1.56*</td>
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<tr>
<td>(5)</td>
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</table>

*) Mole hydrazine formed per minute per mg of protein.
†µg per g of fresh tissue.
Each figure is mean ± S.E. of number of rats given in parenthesis.
\(p < 0.001\) as compared to control.
\(p < 0.01\) as compared to control.
\(p < 0.05\) as compared to control.
\(p < 0.05\) as compared to corresponding Cd group as evaluated by Student's t test.
istered Cd might be related to the intervening role of Cd in the absorption of Zn or to the induction of hepatic metallothionein by Cd.\textsuperscript{10,19} Furthermore, no significant change was observed in the inhibition of renal and hepatic enzymes from the fourth to eighth day following cessation of exposure to Cd. The concentration of renal Cd, however, increased from the fourth to the eighth day, while renal Cu and Zn remained unchanged. Hepatic concentrations of Cd and Zn were almost same at the fourth and the eighth day.

The supplementation of vitamin B-complex concomitantly with Cd reduced most of the enzyme alterations in urine, serum, kidneys, and liver suggesting a protection effect by vitamin B-complex. This protective effect of vitamin B-complex was also shown by the significantly decreased content of renal Cd and Zn on the eighth day, but it was not observed in hepatic levels of these metals. The supplementation of vitamin B-complex reduces alterations in renal, hepatic, and urinary enzymes and accumulation of Pb in blood, liver, kidneys, and brain of Pb intoxicated rats.\textsuperscript{6} Vitamin B\textsubscript{1}, B\textsubscript{12}, and folic acid have been recommended individually in therapeutic doses for the protection of Pb intoxication.\textsuperscript{1,3} The role of vitamin B-complex in ameliorating Cd intoxication may be significant as Cd might cause deficiency of various vitamins in target tissues. The mechanism by which vitamin B-complex counteracts Cd toxicity is not known; however, one or more constituents of vitamin B-complex may interfere with the absorption of Cd in body, possibly by forming readily excretable complexes with Cd or increasing the body resistance against Cd intoxication.\textsuperscript{6}

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References