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Effects of Zinc, Copper, and Lead Toxicity on α-Aminolevulinic Acid Dehydratase Activity

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The distribution of lead, zinc and copper in the human environment has been recognized as a major toxicological factor. Lead ions have been shown to inhibit the activity of δ-aminolevulinic acid dehydratase (δ-ALAD), which is involved in the biosynthesis of heme (Nakao et al. 1968; Haeger-Aronsen et al. 1971; Hernberg and Nikkanen 1972). Copper has also its inhibitory effect on δ-ALAD activity as reported by Gibson and coworkers (1955). A study has shown that the δ-ALAD was activated by zinc ions at physiological concentrations (Meredith and Moore 1978). The δ-ALAD has been considered as bioanalytical tool for environmental lead exposure (European Economic Community 1975; Goyer and Mushak 1977). But the validity of using δ-ALAD inhibition as specific indicator of lead poisoning has been challenged by Thompson et al. (1977). In view of these reports, it was considered worthwhile to study the poisoning effects of lead, zinc and copper on δ-ALAD activity along with the concentrations of these metal ions in the blood. A possible role of Zn^{++}, Cu^{++}, and Pb^{++} interaction and their influence on δ-ALAD has been explored in the present paper.

MATERIALS AND METHODS

Twenty four male abult albino rats of Charles Foster strain weighing 180–200 g were used. The animals were provided with standard laboratory pellet food (Hindustan Lever Laboratory Feeds, India) and water *ad-libitum*. They were maintained in a colony room under constant diurnal conditions of 12-hour (07–19) light and 12-hour (19–07) dark cycle, temperature (25–27° C) and relative humidity (48–50%). The animals were equally divided into four groups. Animals of group I received physiological saline i. p. prepared in demineralized water.

The other II, III and IV groups (experimental) were administered elemental lead acetate 8 mg/kg, zinc acetate 8 mg/kg and copper acetate 8 mg/kg body weight i. p. for 7 consecutive days.

Twenty four hours after the last injection, the animals were anaesthetized with 30 mg/kg of pentobarbitone sodium i. p. The blood samples were collected directly from the heart by means of a heparinized syringe and stored at -4° C.

The δ-ALAD activity was measured in whole blood by the method of Granick and Mausetall (1958). The blood was shaken with an equal volume of 0.1 M phosphate buffer (pH 6.8) and a portion of 0.2 ml sample was incubated at 37° C for 30 minutes. The reaction was commenced by adding 0.4 ml of 3.7 mM δ-aminolevulinic acid in 25 mM phosphate buffer (pH 6.8) with further incubation at 37° C for 60 minutes. The reaction was terminated by adding 1 ml of 4% trichloroacetic acid containing 20 mM $HgCl_2$. After centrifugation, 1 ml of the supernatant liquid was mixed thoroughly with an equal volume of Ehrlich's reagent. The optical density was then recorded at 555 nm.

For the estimation of metal ions, the samples of blood were wet-ashed according to the method of Shafiq-ur-Rehman et al. (1982). Zinc, copper and lead were analysed (Shafiq-ur-Rehman 1983) by an Atomic Absorption Spectrophotometer (PYE-UNICAM-SP-2900).

Data were statistically evaluated employing the Student's *t*-test. Significant comparison was made, between control and experimental groups, considering P at least $\angle 0.05$.

RESULTS AND DISCUSSION

After lead exposure, the concentration of lead ions was increased ($P\angle 0.001$) while zinc ($P\angle 0.05$) and copper ($P\angle 0.01$) levels were decreased. Zinc administration produced increment in its own level ($P\angle 0.001$) but the levels of lead ($P\angle 0.05$) and copper ($P\angle 0.01$) were depressed. Similarly, copper exerted toxic effects by enhancing copper levels ($P\angle 0.001$) and by reducing concentrations of zinc ($P\angle 0.01$) and lead ($P\angle 0.01$). The activity of δ-ALAD was inhibited by the toxic exposure of lead ($P\angle 0.01$) and copper ($P\angle 0.05$), however, zinc activated the enzyme ($P\angle 0.01$)**(Table 1).**

Table 1. Effect of lead, zinc and copper (8 mg/kg body weight i. p. daily for 7 consecutive days) administration on the levels of lead, zinc, copper and δ-ALAD in the blood of rats.

Level	Group I Control (saline)	Group II Lead (8 mg/kg)	Group III Zinc (8 mg/kg)	Group IV Copper (8 mg/kg)
Lead[a]	0.34±0.02	5.60±0.40*** (+1547.06)	0.24±0.02* (−29.41)	0.16±0.01** (−52.94)
Zinc[a]	6.92±0.44	5.10±0.32* (−26.30)	20.12±1.62*** (+190.75)	3.10±0.21** (−55.20)
Copper[a]	1.88±0.14	0.49±0.04** (−73.94)	0.82±0.07** (−56.38)	9.20±1.00*** (+389.39)
δ-ALAD[b]	78.00±5.68	38.0±2.21** (−51.28)	112.0±7.90** (+43.59)	56.0±4.10* (−28.21)

a=Values expressed as μg/ml of blood, ± S. E. of 6 animals.

b=Values expressed as n mol/ml of blood/hr, ± S. E. of 6 animals.

Figures in parentheses indicate % increase (+) or % decrease (−).

Significantly different from control (*=P∠0.05; **=P∠0.01; ***=P∠0.001).

Anemia is regarded as the major effect of heavy metals in humans. It has long been known that the anemia results due to an inhibition of heme synthesis in the blood. The δ-ALAD is one of the principal enzymes involved in the heme synthesis, converting δ-aminolevulinic acid to porphobilinogen and ferrochelatase, and inserting iron into protoporphyrinogen (Vallee and Ulmer 1972). The blockade in the utilization of δ-aminolevulinic acid resulted in subsequent decline in heme synthesis. Lead intoxication has been shown to produce anemia by inhibiting heme synthesis (Leikin and Eng 1963; Hammond 1978). Prasad et al. (1963) have observed that zinc deficiency is responsible for a syndrome of severe anemia. The anemia may also be seen in copper deficiency (Gallagher et al. 1956; Williams et al. 1976). In the present study, the activity of δ-ALAD significantly declined as a result of increased lead content in the blood. A lead-induced depression in the levels of Zn^{++} and Cu^{++} ions was also seen. The overloading consequences (i.e. poisoning) of lead seem to have a direct interaction of Pb^{++} with Zn^{++} and Cu^{++} ions, leading to the formation of an antagonistic association with the two ions. The study is in consonance with earlier studies (Shafiq-ur-Rehman and Chandra 1984; Shafiq-ur-Rehman 1983). It may be possible that anemia produced by lead via depressing δ-ALAD activity is related to an interference of lead with Cu^{++} at absorption level as reported by Klauder et al. (1973). Wilson and coworkers (1972) have shown that one mole of δ-ALAD contains an estimated number of 28 free sulfhydryl (—SH) groups. Since δ-ALAD is a —SH enzyme, its activity is inhibited by a number of heavy metals. Because heavy metals are in dynamic capabilities of reacting with a variety of binding sites especially the functional —SH group to form a most stable metal complexes. Metal ions, however, come into striking consequences when they are heavily overloaded (i.e. intoxicated) beyond their physiological apetite. This phenomena may act by disrupting the normal metal-ligand-equilibrium state to elaborate an impaired-complex system (Shafiq-ur-Rehman 1983). This competitive influence of lead interaction with zinc and copper, initially creates a schism among the metal environment at the particular enzyme process, and then permits the exit of the two metal ions.

It has been recognized that zinc enhances the activity of δ-ALAD (Cheh and Neilands 1973; Abdulla and Haeger-Aronsen 1971). Interestingly, a lead-induced inhibition of the enzyme δ-ALAD was releived by zinc. This protective effect of zinc against toxic manifestation of lead was, for the first time, reported

by Abdulla and Haeger-Aronsen (1971). Another important work was later done which showed that the activity of δ-ALAD was dependent on dietary zinc intake (Finelli et al. 1974), led to propose a functional significance of Zn^{++} to δ-ALAD. Our present finding comes to an understanding with the above report that the activity of δ-ALAD was significantly enhanced following zinc administration. Additionally, the levels of two ions, Pb^{++} and Cu^{++}, were drastically declined as a result of overloaded population of Zn^{++} ions in the blood. This suggests rather a complex (if not a simple one) case because Pb^{++} and Cu^{++} ions were reduced, alleviating their inhibitory influence on δ-ALAD, which provided a chance to Zn^{++} ions to act fully without any hindrance.

Similar to that of lead, the Cu^{++} ions have also shown to be capable of inhibiting the activity of δ-ALAD. Subsequently, the levels of Zn^{++} and Pb^{++} ions were depressed following consistent increase of Cu^{++} ions in the blood. It seems that the inhibitory influence of copper ions on the enzyme δ-ALAD is not either directly or entirely dependent on its own efforts. But it might be acting via displacement of other metal ions from at the enzyme environment. It has been reported that the enhanced copper and depressed zinc and lead levels were related to the hormones, estrogen-progesterone, elevations in the blood (Shafiq-ur-Rehman et al. 1983).

As shown by Klauder and coworkers (1973) that dietary lead caused reduction in plasma copper and ceruloplasmin levels and that the levels of lead in erythrocytes were declined as dietary copper was increased. Similarly, the toxic effect of lead was relieved by dietary zinc (Abdulla and Haeger-Aronsen 1971) via affecting δ-ALAD activity. Whenever one of the Zn^{++}, Cu^{++} or Pb^{++} ions exerts its toxic effect, the other two ions decline significantly in the blood. It appears that each the of three ions, Zn^{++}, Cu^{++} or Pb^{++}, directly strikes at the metal position in the enzyme whereby displacing other metals from —SH binding sites. Since δ-ALAD is a zinc-metalloenzyme, it is apparent that this Zn^{++} ion takes major part as it is required for the enzyme activity. It has been shown that either Pb^{++} or Cu^{++} —induced inhibition of δ-ALAD activity, the Zn^{++} ions were significantly declined. It may be possible that the δ-ALAD inhibition, caused by either lead or copper, is related with zinc metabolism.

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