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Chelation in Metal Intoxication. XIII. Polyaminocarboxylic Acids as Chelators in Lead Poisoning

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Lead intoxication results in an enhanced urinary excretion of lead and δ-aminolevulinic acid (δ-ALA), an inhibition in the activity of blood δ-aminolevulinic acid dehydratase (δ-ALA-D), an increase in lead levels of blood and tissues, and transient hypochromic anemia (HOFMANN & SEGEWITZ 1975; GRANDJEAN 1978; MOLINA-BALLESTEROS et al. 1978; MYKKANEN et al. 1979; ROOTS 1979). The treatment with chelating agents may reduce body Pb by enhancing its excretion and restore at least some of the biochemical alterations (HAMMOND 1971; HOFMANN & SEGEWITZ 1975; TANDON et al. 1981). Calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$, Versenate) has been accepted as a potent antidote for Pb intoxication (CHISOLM 1971; BRIDBORD & BLEJER 1977). However, nephrotoxicity of Versenate and its potentials to quickly mobilize Pb into kidneys in high concentrations and to increase the Pb absorption from gasterointestinal tract, raise serious doubt about its prophylactic use in human Pb poisoning, particularly in cases with renal dysfunction (REUBER & SCHMIELER 1962; CHISOLM 1964; DOOLAN et al. 1967; BRIDBORD & BLEJER 1977). CANTILENA & KLAASSEN (1981) have observed that diethylenetriaminepentaacetic acid (DTPA) followed by EDTA was the most effective among several chelators in enhancing urinary excretion of cadmium, reducing its concentration in various tissues, and preventing acute cadmium intoxication in mice. With a view to have a more useful and safer polyaminocarboxylic acid for controlling Pb poisoning, DTPA, nitrilotriacetic acid (NTA) and hydroxyethylenediaminetriacetic acid (HEDTA) were investigated for their ability to reduce Pb body burden and to restore altered urinary and blood parameters in Pb-poisoned rats.

MATERIALS AND METHODS

Seventy-two female albino rats (180±10 g) of ITRC colony maintained on ad libitum pellet diet (Hind Lever Ltd., India) and water were orally given 10 mg/kg Pb as $Pb(CH_3COO)_2.3H_2O$ (BDH,AR) suspended in distilled water daily for 4 weeks. Twelve animals received an equal volume of water simultaneously, which served as normal control. All the normal controls and 12 Pb-exposed animals were kept in metabolic cages for 24-h urine collection in tubes cooled by ice following last administration before sacrificing for collection of blood, liver, and kidneys.

The remaining 60 Pb-exposed animals were divided into 4 groups and treated intraperitoneally daily for 4 days as follows:

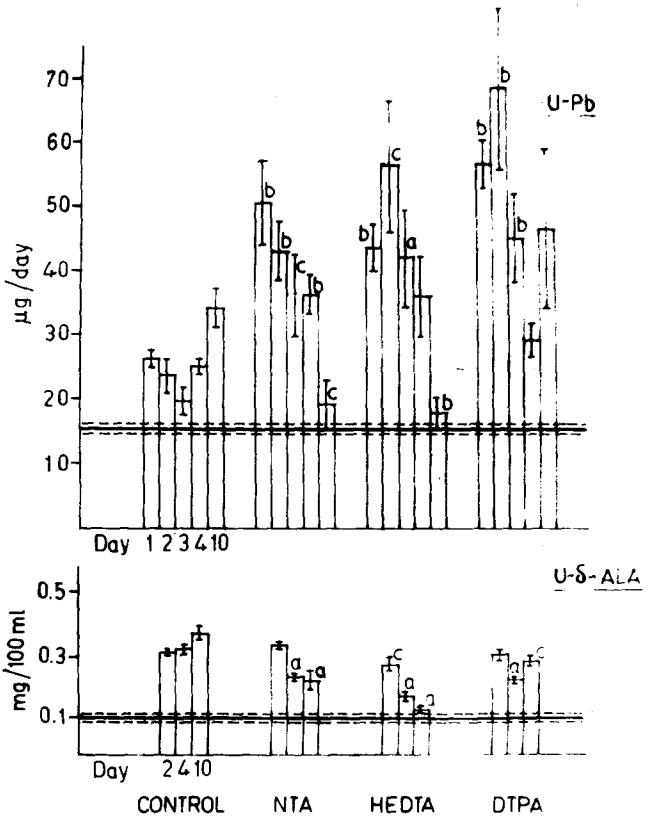

Fig. 1. Effect of polyaminocarboxylic acids on the urinary excretions of lead and δ-aminolevulinic acid in lead-intoxicated rats. Each bar represents mean ± S.E. of 10 to 12 values in control and 5 to 6 values in experimental groups; normal control values represented by horizontal line. $^{a}P<0.001$, $^{b}P<0.01$, $^{c}P<0.05$ when compared to control as evaluated by the Student's "t" test.

Group I - 24 - 0.9% NaCl 4 mL/kg

Group II - 12 - 0.5 mmole/4 mL/kg, NTA (E. Merck)

Group III - 12 - 0.5 mmole/4 mL/kg, HEDTA (Fluka)

Group IV - 12 - 0.5 mmole/4 mL/kg, DTPA (E. Merck)

The injecting solution of NTA, DTPA, or HEDTA was prepared in normal saline with addition of $NaHCO_3$ (pH, neutral). The animals from each group were kept in metabolic cages for 24-h urine collection daily for 4 days during treatment period. Twelve animals from group I and 6 animals from each of the treated groups were sacrificed 24 h after the last injection for collection of blood, liver, and kidneys. The remaining animals were left until the 10th day when 24-h urine was again collected before sacrificing them for blood and tissues.

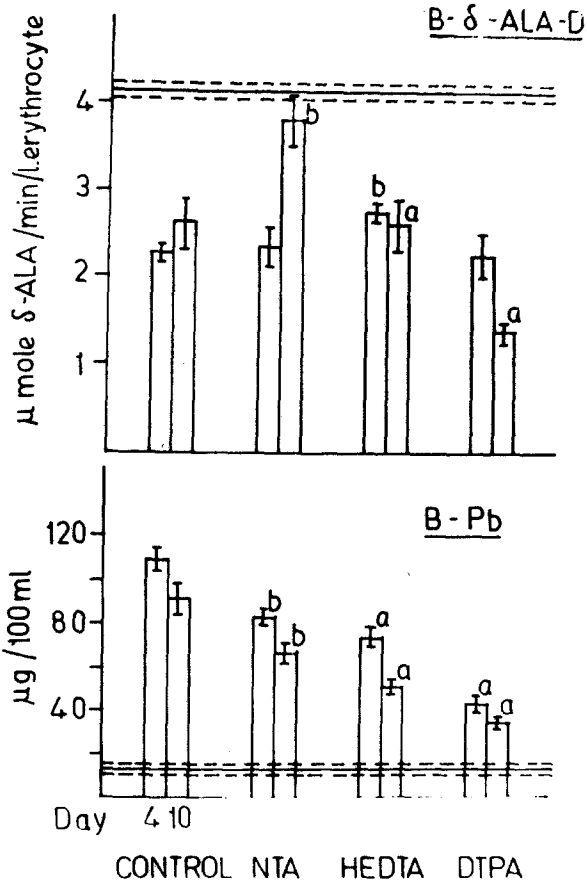

Fig. 2. Effect of polyaminocarboxylic acids on the blood level of lead and activity of δ-aminolevulinic acid dehydratase in lead intoxicated rats. Each bar represents mean ± S.E. of 10 to 12 values in control and 5 to 6 values in experimental groups; normal control values represented by horizontal line. $^{a}P<0.001$, $^{b}P<0.01$ when compared to control as evaluated by the Student's "t" test.

Standard procedures were employed for the estimation of δ-ALA (DAVIS et al. 1968) and Pb (KOPITO & SCHWACHMAN 1967) in urine and for the activity of δ-ALA-D (BERLIN & SCHALLER 1974) and Pb (HESSEL 1968) in blood. The Pb content of liver and kidneys was determined after nitric acid digestion by atomic absorption spectrophotometry (YEAGER et al. 1971).

RESULTS AND DISCUSSION

The oral administration of lead acetate daily for 4 weeks caused a significant increase in the urinary excretion of Pb and δ-ALA, an increase in the Pb levels of blood, liver, and kidneys, and an inhibition in the activity of blood δ-ALA-D indicating Pb intoxication in rats (Table 1).

Table 1. Effect of oral administration of lead acetate on rats.

	Urine		Blood		Liver	Kidney
	(Pb μg/day)	δ-ALA (mg/100 mL)	(Pb μg/100 mL)	δ-ALA-D (μ mole -ALA/min/L erythrocyte)	(Pb μg/g)*	(Pb μg/g)*
Normal control	15.3±0.63 (12)	0.12±0.007 (10)	12.8±1.11 (12)	4.16±0.16 (11)	2.47±0.15 (10)	2.40±0.25 (10)
Lead exposed	32.7±2.19[a] (12)	0.31±0.001[a] (10)	120.0±3.15[a] (12)	2.66±0.24[a] (10)	33.0 ±0.90[a] (12)	19.10±0.71[a] (12)

* Fresh tissue.

Each figure represents mean ± S.E. of the number of values given in parenthesis.

[a] $P < 0.001$, compared to normal control as evaluated by the Student's "t" test.

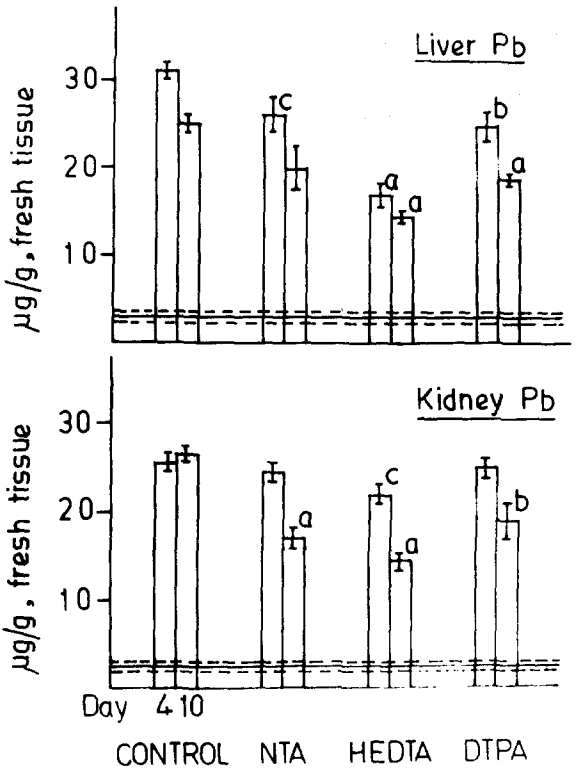

Fig. 3. Effect of polyaminocarboxylic acids on the hepatic and renal levels of lead in lead intoxicated rats. Each bar represents mean ± S.E. of 10 to 12 values in control and 5 to 6 values in experimental groups; normal control values represented by horizontal line. $^{a}P<0.001$, $^{b}P<0.01$, $^{c}P<0.05$ when compared to control as evaluated by the Student's "t" test.

All three chelators were effective in enhancing the urinary excretion of Pb in the poisoned animals; DTPA was superior to HEDTA and NTA. The effect seems to be also lasting with DTPA, as the excretion of Pb remained high even after 10 days of treatment with DTPA while it was lowered significantly in animals exposed to HEDTA or NTA. Concomitantly, DTPA was most successful in lowering the blood-Pb levels. However, HEDTA appears to be more effective than DTPA or NTA in reducing the Pb-induced urinary excretion of δ-ALA and the hepatic and renal levels of Pb, and the levels tended to approach normal with time. The chelating agents showed very little influence on the Pb-induced inhibition in the activity of blood δ-ALA-D and complete recovery in the enzymatic activity could be observed 10 days posttreatment with NTA only (Figures 1 to 3). DTPA and HEDTA have been found to be most effective among several polyaminocarboxylic acids, in checking the toxicity of other metals also by enhancing their excretion and reducing their tissue concentration

(EBYL et al. 1965; TANDON & GAUR 1977; KHANDELWAL et al. 1980; CANTILENA & KLAASSEN 1981; TANDON & KHANDELWAL 1982).

The efficacy of the chelating agents to mobilize toxic metals and restore the altered biochemical parameters may be attributed to the available binding sites in the chelator and the stability constant of excretable metal-chelator complex (KHANDELWAL et al. 1980; TANDON & KHANDELWAL 1982). DTPA possesses 3 amino and 5 carboxyl groups as possible binding sites, and the stability constant of Pb-DTPA complex is reported to be 18.6 followed by HEDTA having 2 amino and 3 carboxyl groups with stability constant of 15.5 for Pb-HEDTA complex (SILLEN & MARTELL 1964). These seem to at least partly explain almost similar magnitude of effect of DTPA and HEDTA which was greater than NTA having only one amino and 3 carboxyl groups with the stability constant of 11.8 for Pb-NTA complex.

Though the present investigation suggests DTPA and HEDTA to be effective alternatives to $CaNa_2EDTA$ in prevention of Pb intoxication, they should be used under strict supervision owing to their toxic side effects particularly nephrotoxicity (DOOLAN et al. 1967; TAYLOR et al. 1974).

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