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INFLUENCE OF ASCORBIC ACID, THIAMINE OR THEIR COMBINATION ON LEAD POISONING IN ALBINO RATS

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Summary

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Thiamine, ascorbic acid and their combination were investigated for their ability to prevent or treat the experimental lead intoxication in rats. The combination of both vitamins was most effective in reducing lead-induced inhibition in the activity of blood δ-aminolevulinic acid dehydratase, elevated urinary excretion of δ-aminolevulinic acid and the uptake of lead in blood, liver and kidney. The combined treatment during and following lead exposure was more effective in restoring lead-induced biochemical alterations and in mobilizing lead from tissues. The order of effectiveness was thiamine + ascorbic acid > ascorbic acid > thiamine.

Key words: ascorbic acid, lead toxicity, prevention, therapy, thiamine, rat

INTRODUCTION

The simultaneous administration of lead and thiamine reduces the uptake of lead in tissues and prevents the clinical signs of lead poisoning in calves (Bratton et al., 1981). The supplementation of vitamin B complex also diminishes lead-induced biochemical alterations in urine, blood, kidney and liver and the tissue accumulation of lead in rats (Flora et al., 1984). Nicotinic acid or a combination of cyancobalamin and ascorbic acid is shown to reverse the inhibition of the *in vivo* heme synthesis by lead (Kao & Fobers, 1973). Dietary supplementation of iron and ascorbic acid prevents lead growth retardation, the reduction in food consumption, anemia, renal hypertrophy and the accumulation of lead in tissues (Suzuki & Yoshida, 1979a, b). Lead poisoning (particularly this one, induced under simultaneous iron deficit) results in development of secondary thiamine insufficiency. Lead intoxication and iron deficiency influence vitamin B group metabolism (Khotimachenko et al., 1977). The efficacy of $CaNa_2EDTA$ to counteract lead intoxication including depletion of brain lead, increases considerably when it is administered in combination with ascorbic acid (Goyer & Cherain, 1979) or thiamine (Flora et al., 1986). These observations suggest a definite role of vitamins in prophylaxis of lead poisoning. Since most of the conventional metal chelating agents may have toxic effects or disadvantages (Doolan et al., 1967; Hammond, 1973; Morgan, 1975; Bridbord and Blejer, 1977; Rosenblatt and Aronsen, 1978; Cantilena and Klaasen, 1982), vitamins may be considered suitable alternative metal antidotes.

In the present study, thiamine hydrochloride (vitamin B_1), ascorbic acid (vitamin C) and their combination were investigated for their potential to prevent or treat the experimental lead intoxication in rats.

MATERIALS AND METHODS

The studies were performed on 90 male Wistar albino rats weighing 190 ± 10 g, maintained on standard pellet diet and drinking water ad libitum. The rats were divided into six groups. Groups I, II (control groups), III, IV ,V consisted of 10 animals each and group VI consisted of 40 animals. Rats were subjected to treatments through gastric gavage 6 days a week once daily, for a period of eight weeks as followed:

- Group I – no treatment;
- Groups II and VI - lead in distilled water[1] – 10 mg Pb/4 ml water/kg (as lead acetate trihydrate[2], Sigma, Germany) + H_2O 4 ml/kg;
- Group III – lead as groups II and VI + thiamine hydrochloride (Sigma, Germany) 25 mg/4 ml water/kg;
- Group IV - lead as groups II and VI + ascorbic acid (Sigma, Germany) 25 mg/4 ml water/kg.;
- Group V - lead as groups II and VI + thiamine (as group III) + ascorbic acid (as group IV).

All rats from groups I-V, were kept in metabolic cages for collection of 24 hours urine. At the end of the 8 weeks treatment, they were sacrificed by decapitation and blood, liver, kidneys and brain were collected.

After the 8-week intoxication, the animals from group VI were subdivided into four equal groups and received the following treatments through gastric gavage, once daily for four days:

- Group VIa – water, 4 ml/kg (intoxicated control group);
- Group VIb – thiamine hydrochloride, 25 mg/4 ml water/kg;
- Group VIc – ascorbic acid, 25 mg/4 ml water/kg;
- Group VId – thiamine hydrochloride as group VIb + ascorbic acid as group VIc.

All mentioned doses are per 1 kg body weight.

Each rat was kept in metabolic cage for collection of 24 hours urine. At the end of the 4-days treatment, ten animals from each group were sacrificed by decapitation and blood, liver, kidneys and brain were collected.

The activity of blood δ-aminolevulinic acid dehydratase (δ-ALA-D) (Berlin and Schaller, 1974) and the urinary excretion of δ-aminolevulinic acid (δ-ALA) (Davis and Abrahams, 1968) were measured using standard procedures. Lead was estimated in blood (Hessel, 1968), liver, kidney and brain specimens (Yeager and Cholak, 1971) by atomic absorption spectrophotometry (Perkin Elmer 5000).

Data were statistically processed using the Student's t-test (Sepetliev, 1986).

RESULTS

The exposure to lead acetate for eighth weeks significantly inhibited the activity of blood δ-ALA-D. The inhibition was less marked in animals simultaneously supplemented with thiamine, ascorbic acid or their combination 3.49 ± 0.17; 4.21 ± 0.11 and 5.04 ± 0.16 µmol δ-ALA/min/l erythrocyte respectively) compared to post exposure treatment groups (3.31 ± 0.07; 4.12 ± 0.15 and 4.75 ± 0.19 µmol δ-

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[1] In all cases of oral treatment of rats, distilled water was used.

[2] $Pb(CH_3COO)_2\ 3H_2O$.

Table 1. Effect of simultaneous supplementation or post exposure treatment with thiamine, ascorbic acid and their combination on lead induced biochemical alterations in rats (mean ± SEM)

Groups		n	Blood δ-ALA-D*	Urine δ-ALA **
Controls	Group I (normal controls)	10	5.93±0.16	0.10 ±0.01
	Group II (intoxication + water)	10	2.82 ±0.14 [a]	0.37 ±0.01[a]
Intoxicated + simultaneously supplemented with vitamins	Group III (thiamine HCl)	10	3.49±0.17 [az]	0.32 ±0.01[ay]
	Group IV (ascorbic acid)	10	4.21±0.11 [az]	0.32±0.01[ay]
	Group V (thiamine HCl+ ascorbic acid)	10	5.04±0.16[bx]	0.25±0.01[ax]
Intoxicated + post exposure treated with water or vitamins	Group VIa (water)	10	2.86±0.30[a]	0.42±0.03[a]
	Group VIb (thiamine HCl)	10	3.31 ±0.07[a]	0.35 ±0.01[a]
	Group VIc (ascorbic acid)	10	4.12±0.15[ay]	0.29±0.02[ay]
	Group VI d (thiamine HCl + ascorbic acid)	10	4.75±0.19[ax]	0.28±0.01[ay]

* µmol δ-ALA/min/l erythrocyte, ** mg/100 ml; [a]P<0.001 compared to normal control, [x]P<0.001, [y]P<0.01, [z]P<0.05 compared to the respective water treated group.

ALA-D/min/l erythrocyte respectively). The activity of the enzyme recovered to a higher degree after treatment with ascorbic acid or thiamine plus ascorbic acid for four days (Table 1). The inhibition of blood δ-ALA-D activity resulted in enhanced urinary excretion of δ-ALA. The increase in δ-ALA excretion was however, less marked in animals concomitantly administered thiamine, ascorbic acid or their combination (0.32 ± 0.01; 0.32 ± 0.01 and 0.25 ± 0.01 δ-ALA mg/100 ml respectively) compared to post exposure treatment groups (0.35 ± 0.01; 0.29 ± 0.02 and 0.28 ± 0.01 δ-ALA mg/100 ml respectively) excepting ascorbic acid treated groups. The lead induced excretion of δ-ALA was lowered significantly following treatment with ascorbic acid or its combination with thiamine (Table 1). Differences were significant versus normal control or to water treated groups. The treatment with thiamine alone was ineffective in both restoring the inhibited activity of blood δ-ALA-D and in lowering the urinary δ-ALA excretion (Table 1).

The uptake of lead by blood and kidneys was prevented at a higher extent in animals, simultaneously supplemented with thiamine, ascorbic acid or their combination (90.8 ± 5.1; 73.25± 3.52 and 54.10 ± 4.23 mg Pb/100 ml blood respectively) compared to post exposure treat-

Table 2. Effect of simultaneous supplementation or post treatment with thiamine, ascorbic acid and their combination on blood and tissue level of lead in rats (mean ± SEM)

Groups		n	Blood *	Liver **	Kidney**	Brain**
Controls	Group I (normal controls)	10	9.12 ± 0.39	0.75 ± 0.09	0.91± 0.08	0.32 ± 0.04
	Group II (intoxication + water)	10	116.8± 7.20^{a}	9.59 ± 0.73^{a}	9.71± 0.41^{a}	4.94 ± 0.30^{a}
Intoxicated + simultaneously supplemented with vitamins	Group III (thiamine HCl)	10	90.80 ±5.10^{az}	8.41 ± 0.89^{a}	7.55± 0.55^{az}	5.60 ± 0.43^{a}
	Group IV (ascorbic acid)	10	73.25 ±3.52^{ax}	6.85 ± 0.49^{az}	5.69 ± 0.29^{ax}	5.53 ± 0.20^{a}
	Group V (thiamine HCl+ ascorbic acid)	10	54.10 ± 4.23^{ax}	5.39 ± 0.39^{az}	4.38 ± 0.21^{ax}	5.62 ± 0.23^{a}
Intoxicated + post exposure treated with water or vitamins	Group VIa (water)	10	136.07 ±5.90^{a}	7.01 ± 0.43^{a}	11.05± 0.41^{a}	3.58 ± 0.12^{a}
	Group VIb (thiamine HCl)	10	105.40 ±4.03^{ay}	5.01 ± 0.33^{ay}	9.20 ± 0.27^{ay}	4.06 ± 0.35^{a}
	Group VIc (ascorbic acid)	10	93.99 ± 4.33^{ax}	4.00 ± 0.22^{ax}	6.88 ± 0.12^{ax}	4.21 ± 0.32^{a}
	Group VI d (thiamine HCl + ascorbic acid)	10	85.56± 5.56^{ax}	4.01 ± 0.22^{ax}	5.90 ± 0.29^{ax}	3.45 ± 0.12^{a}

*mg/100ml, **µg/g fresh tissue; ^{a} $p<0.001$ compared to control group, ^{x} $p<0.001$, ^{y} $p<0.01$, ^{z} $p<0.05$ compared to the respective water treated group.

ment groups (105.4 ± 4.03; 93.99 ± 4.33 and 85.56 ± 5.56 mg Pb/100 ml blood respectively). The content of lead in liver was relatively lower in post exposure treatment groups. The treatment of lead exposed animals with these vitamins, caused a significant elimination of lead from blood, liver and kidney compared to the water-treated group (Table 2). However , the vitamins could neither prevent the uptake of lead by brain nor could mobilize the metal from this tissue (Table 2).

The simultaneous supplementation with thiamine, ascorbic acid or their combination was more effective in preventing lead-induced biochemical alterations, blood and tissue uptake of lead than the treatment with these vitamins in restoring the changes caused by lead and its mobilization from the tissues. The order of effectiveness was thiamine+ascorbic acid > ascorbic acid > thiamine.

DISCUSSION

Ascorbic acid (Goyer and Cherian, 1978) or thiamine (Flora et al., 1986) greatly enhanced the prophylactic potential of CaNa$_2$EDTA in lead intoxication including removal of lead from the central nervous system. The combination of ascorbic acid and CaNa$_2$EDTA was also effective in removing intranuclear lead inclusions from the kidney (Goyer and Cherian, 1978). Lead exposed workers, maintained on therapeutic regimen of ascorbic acid plus zinc, showed a significant decrease in blood lead concentration (Papaioannu and Sohler, 1978). The protective or therapeutic actions of vitamins may be directed via the formation of readily excretable complex(es) of lead with vitamins or their metabolites or indirectly via the modification of the antidotal properties of the complementary chelating agent (Bratton et al.,1981). The concept of vitamin B_1-Pb complex is more realistic if one would consider a conformational transformation of thiamine molecule (one form with closed thiazole ring and two forms with open thiazole ring). The occurrence of such forms of vitamin B_1 has been shown *in vitro* (Metzier and Maier, 1962) and they apparently occur *in vivo*, but in very small quantities. In the open thiazole ring conformation, the exposure of SH groups in vitamin B_1 molecules permits several possible configurations of vitamin B_1-Pb complexes (Olkowski et al., 1991).

There is dynamic equilibrium between open ring and closed ring forms of vitamin B_1 in the intracellular compartment , but the concentration of open ring forms is low. However, if upon administration of vitamin B_1 in high doses the concentration gradient creates an inwardly directed continuous movement of vitamin B_1, then the intracellular environment would become more pronounced (Olkowski et al.,1991)

Lead intoxication causes deficiency of thiamine (Tokarski and Reio,1978) and alterations in ascorbic acid metabolism (Rudrapal et al., 1975). Accordingly, supplementation with these vitamins during lead intoxication might alleviate lead-induced vitamin deficiency or disturbance in their metabolism.

The present results showed that ascorbic acid is more effective than thiamine whereas their combination is more effective than either of them individually in both prevention and treatment of lead intoxication. The ascorbic acid is reported to act as a detoxifying agent by forming poorly ionized, but soluble compound with lead (Pillemer and Seifter, 1940). It is not a carboxylic acid but a lactone containing an enediol group.

It is likely that one of OH group from the enediol group and C=O from two moles of ascorbic acid participate in forming a chelate ring with Pb. Since the absorption of ascorbic acid from the gastrointestinal tract following gastric gavage is higher than that of thiamine (Goodman and Gilman, 1975), the former is apparently more effective in complexing lead. The relatively higher efficacy of ascorbic acid may also be attributed to its *in vivo* ability to reduce the disulfides to SH-containing compounds required for the stimulation of heme synthetase activity (Kao and Forbes, 1973).

However, the combination of ascorbic acid and thiamine may be regarded as complementary lead complexing agent with mainly alleviating effects.

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