INFLUENCE OE CALCIUM DISODIUM EDETATE ON THE TOXIC EFFECTS OF LEAD ADMINISTRATION IN PREGNANT RATS

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Summary : Adverse effects of lead on mothers and foetal development alongwith lead distribution and their alteration by calcium disodium ethylenediamine tetraacetate ($CaNa_2EDTA$) were investigated in pregnant rats. The number of fetal resorption and abnormal fetuses increased and the number of live fetuses per dam and fetal weight decreased alongwith increase in liver, kidney, blood, brain and fetus lead levels by lead administration. The treatment with $CaNa_2EDTA$ significantly reduced these effects of lead. However, the chelating agent enhanced the placental level of lead. Neither lead nor $CaNa_2EDTA$ altered the zinc levels of maternal organs, placenta or fetus. The results suggest that many adverse effects of lead in pregant rats can be favourably reduced by $CaNa_2EDTA$.

Key words : lead teratogenicity $CaNa_2EDTA$ pregant rat

INTRODUCTION

Chronic exposure to lead may result in spontaneous abortion and fetal death (1,2). Lead has been shown to cross the placental barrier in experimental animals (3) and in man (4) However, the placental transfer is relatively slow and the levels in fetal tissues never reach equal to those in maternal organs at least after a single dose to mother (5). The administration of lead during the susceptible period induces rather specific effects on the tail buds of hamster embryos (6).

Chelating agents form complexes with various metals and can alter the pattern of distribution and excretion of metals; thus they may be useful in the treatment of metal intoxication. The prolonged administration of EDTA produced malformation in rats (7). Thus, chelating agents may augment the effects of teratogens of complexing the toxic metal. Swenerton and Hurley (8) showed that zinc supplementation in maternal diet prevented EDTA-malformations suggesting that these might be caused by zinc deficiency. In the present study, teratogenic and other effects of lead and the influence of $CaNa_2$ EDTA on such effects were investigated in pregnant rats.

MATERIAL AND METHODS

Female albino rats (225-250 g) were mated overnight. The following morning was designated as day 1 of gestation if a vaginal plug was found. The pregnant animals were housed in air-conditioned room and had free access to water and pellet diet. They were divided equally into 7 groups of six each and were treated as follows :—

Group 1 - No treatment (control); Groups 2 to 5-5, 10, 20 or 40 *mg/kg* lead as lead nitrate, dissolved in distilled water, iv, on day 9, 10 and 11 of gestation; Group 6-0.3 *mmol/kg* $CaNa_2EDTA$ dissolved in distilled water ip, twice on day 13 of gestation; group 7-40 *mg/kg* lead as in group 5 followed by 0.3 *mmol/kg* $CaNa_2EDTA$ as in group 6.

On day 22 of gestation, the rats were killed in gaseous carbon dioxide. Maternal organs and fetuses were collected and blood was sampled in heparinized vials. The number of live, dead and resorbed fetuses and implantation sites were recorded. Fetuses were weighed and examined for external malformation. The kidney, liver, brain, placenta and whole fetuses were digested with conc. HNO_3 for the estimation of lead (283.3 nm) and zinc (213.9) by atomic absorption spectrophotometer (Perkin Elmer 5000) following standard procedures (9,10). Lead and zinc contents of blood were estimated according to the methods of Hessel (11) and Parker *et al.* (10), respectively.

RESULTS AND DISCUSSION

The administration of 10, 20 or 40 *mg/kg* but not 5 *mg/kg* of lead (given iv, for 3 days) produced observable maternal toxicity. The administration of $CaNa_2EDTA$ alone (0.3 *mmol/kg*, ip) produced no gross toxic effects except that the rats became anorexic, lethargic and showed slight diarrhea. These effects persisted throughout the rest of gestation. The weight gain was less, though not significant in pregnant rats given 10, 20 or 40 *mg/kg* lead compared to control. One maternal death was noted on day 13 of gestation following treatment with 40 *mg/kg* lead. There was one dam with all fetuses reabsorbed among animals exposed to 20 or 40 *mg/kg* lead. The resorption per dam also increased with the dose of lead. There were significantly fewer live births per dam for doses at 10, 20 or 40 *mg/kg* lead as compared to control. However, the treatment with $CaNa_2EDTA$ produced a significant decrease in the number of resorption per dam and increase in number of live fetuses in lead (40 *mg/kg*) exposed mothers. The mean fetal body weight decreased with increase in dose of lead. The fetal body weight sightly increased on treatment with $CaNa_2EDTA$ in lead (40 *mg/kg*) pre-exposed rats. The incidence of birth of abnormal pups (with external abnormalities, particularly, tail malformation) was significantly greater in rats exposed to 10, 20 or 40 *mg/kg*, lead as compared to control. The treatment with $CaNa_2EDTA$ had no marked effect on lead (40 *mg/kg*) induced malformations in fetuses (Table I).

TABLE I : Maternal and fetal observations showing effects of lead and $CaNa_2EDTA$ treatment.

	Control	*$CaNa_2EDTA$*	*Lead (mg/kg)*			*Pb (40 mg/kg)-$CaNa_2EDTA$*
			10	*20*	*40*	
No. dams treated	6	6	6	6	6	6
No. Maternal death	0	0	0	0	1	0
No. dams with all fetuses reabsorbed	0	0	0	1	1	1
Weight at start of experiment (g)	220.7±5.7	211.3±6.3	211.3±4.1	212.4±6.3	209.4±7.3	215.3±2.7
Weight gain (g)	25.2±1.2	26.1±5.7	18.4±3.2	18.2±3.2	12.2±2.7[a]	14.4±2.7[a]
Implants/dam	12.6±0.6	11.4±0.4	10.1±2.1	11.3±0.7	11.6±0.8	11.9±0.7
Resorption/dam	0.21±0.04	0.23±0.04	2.3±0.7[b]	4.91±0.19[a]	7.15±0.19[a]	3.11±0.41[a]**
Live Fetuses/dam	12.4±0.4	11.2±0.2	7.8±1.9	6.4±0.4[a]	4.4±0.6[a]	8.8±0.5[c]**
Fetal Body Weight (g)	5.0±0.4	4.1±0.2	4.1±0.2	3.0±0.5[c]	2.7±0.5[b]	3.8±0.1[c]
No. abnormal fetuses/dam	1.00±0.02	2.13±0.80	2.76±0.01[a]	3.01±0.01[a]	3.4±0.08a	3.01±0.17[a]

Each value is expressed as mean±SE of 6 animals
[a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$ versus control; **$P<0.01$ versus lead (40 *mg/kg*) exposed group, as evaluated by Student's 't' test.

TABLE II : Effect of lead and $CaNa_2EDTA$ treatment on the lead levels in various maternal organs and whole fetus.

	Control	*$CaNa_2EDTA$*	*Lead (mg/kg)*				*Pb (40 mg/kg) $CaNa_2EDTA$*
			5	*10*	*20*	*40*	
Liver †	1.07±0.07	0.83±0.13	4.12±0.12[a]	6.39±0.36[a]	12.63±1.43[a]	19.59±3.17[a]	6.21±0.63[a]***
Kidney †	0.51±0.29	0.43±0.04	1.37±0.12[c]	2.41±0.10[b]	5.23±0.13[a]	8.15±0.51[a]	6.13±0.20[a]*
Brain †	N.D.	N.D.	0.13±0.02[a]	0.29±0.01[a]	0.34±0.04[a]	0.35±0.01[a]	0.40±0.03[a]
Placenta †	N.D.	N.D.	0.19±0.02[a]	0.31±0.01[a]	0.37±0.04[a]	0.36±0.04[a]	0.53±0.02[a] *
Blood ††	4.13±0.61	5.4±0.61	10.21±0.61[a]	13.13±0.27[a]	29.41±0.41[a]	45.03±0.31[a]	18.23±0.79[a]***
Fetus †	N.D.	N.D.	0.21±0.04[a]	0.18±0.02[a]	0.36±0.01[a]	0.46±0.02[a]	0.40±0.02[a]

† *μg/g* fresh tissue; †† *μg/100 ml.*

Each value is mean±SE of 6 animals. [a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$ versus control;

***$P<0.001$, *$P<0.05$ versus lead treated (40 *mg/kg*) group as evaluated by the student 't' test.

The lead levels of liver, kidney, brain, blood and whole fetus increased significantly with the dose of lead. The lead concentration of placenta did not differ with the dose of lead. The treatment with $CaNa_2EDTA$ of lead (40 *mg/kg*) administered rats, decreased lead concentration of liver, kidney and blood, while the placental level increased significantly. The brain and fetal concentration of lead however, remained practically unaffected by $CaNa_2$ EDTA treatment (Table II). The zinc concentration of all the maternal organs and fetuses remained uninfluenced by lead, $CaNa_2EDTA$ or lead-$CaNa_2EDTA$ treatment (Control values - Liver 40.13 ± 4.19; Kidney 22.47 ± 6.01; Brain 18.49 ± 3.17; Blood 10.41 ± 1.03; Fetus 14.07 ± 2.14; Placenta 9.34 ± 3.10).

Lead produced a definite dose dependent increase in teratogenic effects. The result also showed that treatment with $CaNa_2EDTA$ reduced the number of resorption per dam and increased the number of live fetuses in lead treated mothers. It is possible that lead - $CaNa_2$ EDTA complex could reach an otherwise inaccessible site in the embryo or fetus and produce qualitatively different toxic response (12). However, this was not apparent as all malforomations were chracteristic of lead in lead - $CaNa_2EDTA$ treated group.

The treatment with $CaNa_2EDTA$ of lead exposed pregnant rats showed marked influence on the distribution of lead in maternal organs as observed by a decrease in liver, kidney and blood lead levels. These results are similar to those observed in non-pregnant rats (13,14). The prolonged administration of EDTA has been shown to produce malformations in rats (7). However, no significant effects were observed in the present study probably due to the short duration of $CaNa_2EDTA$ administration.

The results demonstrate that lead injected intravenously crosses placenta and is detected in fetuses in rats and that the ratio of placental to fetal lead concentration is not related with the dose.

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