THE PROTECTIVE ROLE OF L-CYSTEINE AGAINST FOLLICULAR ATRESIA INDUCED BY LEAD IN MOUSE OVARY

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Abstract- Lead is an ubiquitous environmental toxin that induces a broad range of physiological biochemical, and behavioral dysfunctions. In this study, we examined the pathologic effects of lead acetate in NMRI mouse ovarian tissue and the protective role of antioxidant L-cysteine, against the induced damage. We used lead acetate at a dose of 10 mg/kg, and L-cysteine at a dose of 200 mg/kg. Both drugs were administered intraperitoneally according to 2 protocols: intraperitoneal injection of lead acetate 10 mg/kg/day for 15 days or 10 mg/kg/week for 15 weeks. Ovaries were examined histologically and changes in the number of graafian, growing, atretic, and primordial follicles, thickness of granolusa of theca layers, relative ovary weight (ROW) and animal weights, were determined. Significantly increased numbers of atretic follicles and thickness of the theca layer, and a decrease in other parameters were observed after treatment with lead acetate ($P< 0.05$). No changes were observed after treatment with a combination of L-cysteine. Also, more oocytes had resumed meiosis in the follicles exposed to lead acetate. The results suggest that lead acetate at a dose of 10 mg/kg has a toxic effect on ovarian tissue, and antioxidants such as L-cysteine have a protective role against the induced damage.

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Key words: L-cysteine, protective, lead acetate, ovary, atretic follicle, mouse

INTRODUCTION

Lead is a pervasive environmental pollutant whose mechanism of toxicity is currently being investigated. Experimental studies on animals have shown that low levels of lead accumulation in the ovaries could impede folliculogenesis (1). There is damage to primordial follicles and inhibition of follicular development in chronically lead-intoxicated Rhesus monkeys (2) and rats (3). Although no single mechanism for lead toxicity as yet has been defined, recent studies suggest that at least some lead-induced damages may occur as a consequence of its propensity for disrupting the delicate pro-oxidant/antioxidant balance that exists within mammalian cells (4). Containment of the damage associated with escalating oxidative stress in lead-exposure may be achieved through administration of synthetic antioxidant compounds such as L-cysteine. Heavy metals have properties that mechanically effect antioxidant processes. The pro-oxidant properties of these metals are exacerbated by their inhibitory effects on antioxidant processes. Lead and other heavy metals have high affinities for glutathione (GSH), which is the primary intracellular antioxidant and conjugation agent for an excellent review of GSH metabolism (5). There are very few published studies on the amount of lead in ovaries.

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We have examined the accumulation of lead in the ovary and the protective role of L-cysteine against follicular atresia induced by lead in mouse ovary. For this purpose, we have used two exposure protocols in order to study the same cumulative dose over different exposure periods. We have also checked whether low levels of lead accumulation in the ovaries could impede folliculogenesis.

MATRERIALS AND METHODS

Eight-week-old and five-week-old female mice (NMRI, Pasteur Institute of Iran) were exposed to lead according to the two following protocols: 1)acute- intraperitoneal (IP) injection of lead acetate 10 mg/kg body weight/day for 15 days 2) chronic- IP injection of lead acetate 10 mg/kg body for 15 weeks (Table 1).

For protective experiments, mice in both the acute and chronic groups were exposed to L-cysteine and lead, (IP. injection of lead acetate 10 mg/kg body weight and IP. injection of L-cysteine 200 mg/kg body weight). IP. injection was chosen for better control of the cumulative doses (14). Both the acute and chronically treated mice received the same cumulative quantity of lead. Mice were maintained under standard animal house conditions (temperature 25±2ºC, relative humidity 50±5%) with a regularly alternating cycle of 12 h light and darkness. All females were weighed at the beginning and at the end of the treatment (Table 1). At the end of the exposure, 48h after the end of injection, all mice were sacrificed and ovaries from control, acute and chronically exposed and protected (L-cysteine and Lead) groups were fixed in Bouin's fluid for one day. Sections (6µm) were cut and stained with hematoxylin-eosin. The follicles in every sixth serial section were counted and classified according to Pedersen (6) into primordial follicles (an oocyte surrounded by a single layer of granulosa cells, and a diameter < 100µm), growing follicles (an oocyte surrounded by several layers of grandulosa cells, without an antrum, and a diameter of 100 to 300 µm) or graafian follicles (a peripheral oocyte surrounded by cumulus cells and several layers of granulosa cells, an antrum and a diameter> 300 µm). Only those follicles in which the nuclei or germinal vesicles of the oocytes were visible in the section were counted. All sections were scored and results were expressed as percentages of primordial, growing, graafian and atretic follicles. The quality of the follicles was taken into account; they were classified as healthy follicles (without pyknotic cells) or atretic ones (with pyknotic cells).

Table 1. Protocol for acute and chronic lead intoxication and group

Administration scheme	Mice age at the time of experiment	Total
Acute		
Control		
Sodium acetate 15 days 10 mg/kg/IP/day during		3 series of 10 mice
Lead acetate 15 days 10 mg/kg/IP/day during		3 series of 10 mice
L-cysteine 15 days 200 mg/kg/IP/day during	8 week-old	3 series of 10 mice
Lead acetate+ 10 mg/kg/IP/day during		3 series of 10 mice
L-cysteine 15 days 200 mg/kg/IP/day during		3 series of 10 mice
Chronic		
Control		
Sodium acetate 15 weeks 10 mg/kg/IP/every week		3 series of 10 mice
Lead acetate 15 weeks 10 mg/kg/IP/every week		3 series of 10 mice
L-cysteine 15 weeks 200 mg/kg/IP/every week	5 week-old	3 series of 10 mice
Lead acetate+ 10 mg/kg/IP/every week		3 series of 10 mice
L-cysteine 15 weeks 20 mg/kg/IP/every week		3 series of 10 mice

The follicles were classified as atretic when more than 10 pyknotic cells were observed per section (resumed meiosis in the nuclei of oocytes in atretic follicles). The weights of control and experimental groups were compared using the t-test and the level of significance was established at $p< 0.05$.Continuous variables were compared using the Bonferroni ANOVA test followed by a Dunn's multiple comparisons test; differences were considered significant at $p< 0.05$. All statistical analyses were carried out using SPSS software. The percentages of oocytes at different histologic stages were compared using the Chi-square test, significant with $p< 0.05$.

RESULTS

The mean body weights of control and experimental animals at the beginning of the experiments were not similar, and there were significant differences in the weight gains of control and lead-treated mice during the experiments ($p< 0.05$), but no significant differences were observed between control and protected groups with acute and chronic exposure regarding the weight of ovaries (Table 2).

Table 2. Total body and ovary weights in mice acutely or chronically intoxicated with lead acetate and protected group

	Mean body weight (g)		Mean organs weight (mg)
	At start	At sacrifice	ovary
Acute:			
Control	24.5±2.6	28.4±2.7	8.6±2.1
Sodium acetate	23.6±2.2	28.1±2.4	8.2±1.8
L-cysteine	24.0±2.4	28.0±2.2	8.3±2.6
Lead	23.1±2.1	23.9±1.6	7.8±3.1
Lead + L-cysteine	23.3±2.2	25.9±1.1	8.0±2.2
Chronic:			
Control	16.5±1.0	27.5±2.2	17.6±4.3
Sodium acetate	16.1±0.6	27.3±3.1	17.4±4.1
L-cysteine	15.9±0.6	26.9±1.3	17.1±4.0
Lead	15.2±1.0	21.8±1.0	10.4±2.1
Lead + L-cysteine	16.6±0.7	24.1±1.5	14.9±3.4

Data are expressed as mean± SD.
There are significant differences in body weights before and after treatment and ovary weight between control and treated groups (t-test, $p< 0.05$, n= 300).

The percentage of primordial follicles in the acute treatment group showed no significant difference between control and treated groups, while the percentage of primordial follicles in the chronic treatment group showed a significant difference between control and lead-exposed mice ($p< 0.05$), but no significant difference was seen between control and protected group.

There was a significant difference in the percentage of growing follicles per ovary among control and lead-exposed mice ($p < 0.05$), with no significant difference between the percentages of growing follicles in ovaries of control and protected groups. The percentage of graafian follicles was significantly lower than in control ovaries, while the percentage of atretic follicles ($p< 0.05$) and thickness of theca layer were greater in the control group ovaries ($p< 0.05$, Table 3).

Also, a significant difference was seen in the thickness of the granolusa layer per ovary of control and lead-exposed mice ($p< 0.05$), with no significant difference in the thickness of the granolusa layer between control and protected group (Table 4).

Table 3. Effect of protective L-cysteine on number of primordial, growing, graafian and atretic follicles in lead-exposed mice

	Primordial follicle	Growing follicle	Graafian follicle	Atretic follicle
Acute:				
Control	47.5±4.2	21.5±0.9	3.8±0.6	0.5±0.1
Sodium acetate	46.8±3.3	20.3±0.6	3.7±0.5	0.6±0.1
L-cysteine	46.5±3.1	21.0±0.5	3.8±0.4	0.5±0.1
Lead	44.1±2.9	12.9±0.1	1.8±0.1	1.3±0.2
Lead + L-cysteine	45.3±3.0	18.8±1.2	3.2±0.3	0.8±0.1
Chronic:				
Control	48.7±4.5	20.6±1.2	4.0±0.3	0.5±0.1
Sodium acetate	47.9±3.8	19.8±0.9	3.9±0.2	0.6±0.1
L-cysteine	47.6±3.4	20.1±0.8	3.9±0.2	0.5±0.1
Lead	35.9±2.9	11.5±1.5	1.1±0.1	2.0±0.2
Lead + L-cysteine	42.9±3.3	17.9±0.3	3.4±0.3	0.9±0.1

Data are expressed by mean±SD.
There are significant differences in number of primordial, growing, graafian and atretic follicles before and after treatment between control and treated groups (t-test, $p< 0.05$, n= 300).

More oocytes had resumed meiosis in the follicles of lead-exposed animals than in those of the control group. The ovaries of the control mice at various stages of growth, were healthy (less than 2.1% were atretic). Representative photographs of early antral follicles and oocytes enclosed in pre-ovulatory follicles are shown in Figure 1. Whereas control ovaries contained healthy antral follicles (Fig. 1A), the ovaries from lead-exposed mice contained atretic antral follicles, with detached granulosa cells, pyknotic nuclei in the granulosa wall and a hypertrophic theca layer (Fig. 1B). The oocytes enclosed in healthy antral follicles showed a typical perinuclear chromatin condensation (Fig. 1C). Most of the oocytes enclosed in atretic pre-ovulatory follicles had resumed meiosis; and the oocytes had a metaphase I (as shown on Fig. 1B) or a metaphase II plate and were often degenerated. There were significant differences between control and treated groups, in the thickness of theca and granolusa layers before and after treatment (Student t-test, $p< 0.05$, n= 300).

Table 4. Effect of protective L-cysteine on thickness of theca and granolusa layer in lead-exposed mice

	Thickness of theca layer (µm)	Thickness of granolusa layer (µm)
Acute:		
Control	16.9±1.3	63.1±4.5
Sodium acetate	16.7±1.2	62.2±3.9
L-cysteine	16.3±1.3	63.4±4.1
Lead	22.3±0.9	48.6±2.9
Lead + L-cysteine	18.5±1.8	59.9±3.3
Chronic:		
Control	15.5±2.5	64.7±5.5
Sodium acetate	15.3±2.1	63.2±4.9
L-cysteine	15.4±2.4	64.1±4.6
Lead	24.2±1.1	42.2±3.4
Lead + L-cysteine	19.8±2.6	56.6±5.1

Data are expressed by mean±SD.

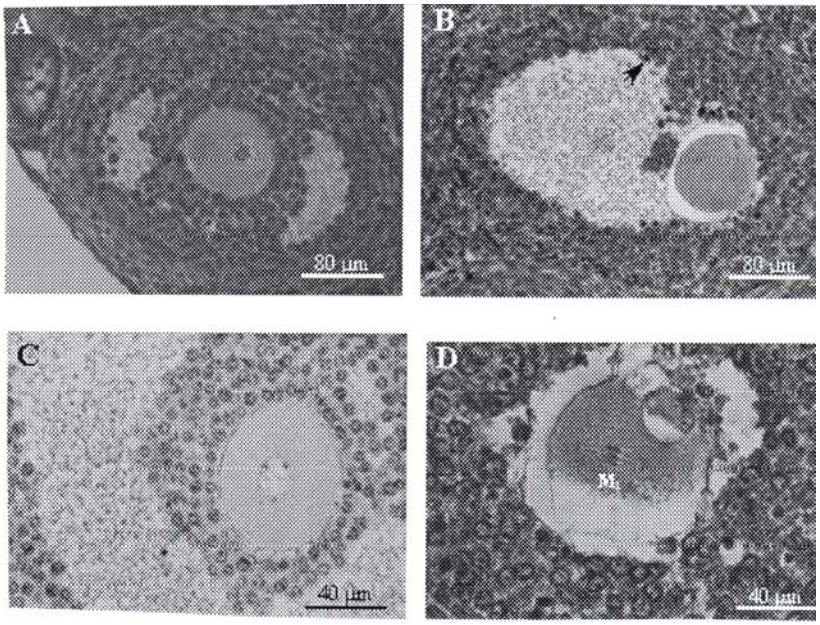

Fig. 1. Histology of early antral follicles and oocytes observed in ovarian sections from control and chronically intoxicated mice. Healthy early antral follicle from a control mouse: oocyte surrounded by cumulus cells (A); Atretic early antral follicle from a chronically lead-intoxicated mouse: inside is enclosed a denuded oocyte which is already at the metaphase I stage (B); Cumulus-enclosed oocyte observed in an healthy follicle with a large antrum: it shows a typical perinuclear chromatin condensation (C); A denuded oocyte observed in an atretic follicle with a large antrum: it has precociously resumed meiosis and is at the metaphase I (MI) stage (D); note the presence in (B) and (D) of pyknotic cells in the granulosa layer and, free at the periphery of the antrum (arrow) (B, D).

DISCUSSION

The ovary plays a pivotal role in reproduction, as the development, maturation, and ovulation of the female gametes occur within the ovarian follicles. Many studies suggest that lead causes direct damage to the ovaries, resulting in ovarian follicular cysts and fewer corporea lutea at high lead concentrations (7). These studies provide no dose-effect information. As exposure to high lead concentrations causes considerable damage to mouse ovaries (8), we have focused on whether low concentrations inhibit folliculogenesis. This possibility is supported by the small number of corpora lutea observed in lead-intoxicated rats (7). There is also a reduction in the number of small and medium follicles following low lead-acetate intoxication and fewer large follicles with high concentrations of lead acetate in mice (8), which are in agreement with our results. There is also damage to primordial follicles and inhibition of follicular development in chronically lead-intoxicated Rhesus monkeys (9) and rats (10). Our histologic studies showed changes in the follicular cells and oocytes of chronically lead-intoxicated mice, with disruption of the follicular membrance, increased pyknosis in granulosa cells, and hypertrophy of the theca layer, indicating follicular atresia. Lead treatment also increased the number of oocytes that had resumed meiosis, in agreement with the fact that follicular atresia is responsible for the resumption of meiosis and oocyte degeneration in several mammalian species (11,12). Whatever the intoxication protocol, mating was sometimes followed by gestation, but the total rate was very low. Lead intoxication does not cause total sterility, but it disturbs the fertility of the female.

Lead and other heavy metals have high affinities for GSH, which is the primary intracellular antioxidant and conjugation agent (13). Lead-induced depletion of intracellular GSH and increased levels of malondialdehyde in ovary and other organs, also inhibits the activities of two key enzymes involved in GSH metabolism: GSH synthetase and GSH reductase (14). Lead also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase, and perhaps GSH peroxidase (15). The presence of a sulfhydryl group in the structure of L-cysteine gives it the potential to function as a chelating agent (16). An adaptive and protective response to toxic metal exposure is induction of metallothionein (MT) synthesis. Metallothioneins are low-molecular-weight metal-binding proteins with 61068 amino acids, 20 of which are represented by cysteine residues in conserved positions (17). MTs play a pivotal role in metal-related cell homeostasis due to their high affinity for metals, in particular zinc and copper for cell growth and development, and lead and cadmium to avoid the toxicity of the latter two metals (18). Twenty cysteine amino acids are found in reduced form and bind seven zinc atoms through mercaptide bounds forming metal thiolate clusters (17). In this peculiar antioxidant task, MTs are also transferred by means of chaperones from the cytosol into the nucleus to protect DNA from oxidative and chemical damage, as well as from DNA fragmentation (apoptosis), the importance of metallothionein in the protection against toxic metals is evident (19). However, our data demonstrate that even very low lead levels in this organ are able to disturb cogenesis. The results suggest that lead acetate at a dose of 10 mg/kg has a toxic effect on ovarian tissue and antioxidants such as L-cysteine have a protective role against the damage.

REFERENCES

1. Lefevre B. Lead accumulation in the mouse ovary after treatment-induced follicular atresia. Reproductive Toxicology 2001; 15: 385-439.

2. Vermande Van Eck GJ, Meigs JW. Changes in the ovary of Rhesus monkey after chronic Lead intoxication. Fertil Steril 1960; 11: 223-234.

3. Stowe HD, Moore RA. The reproductive ability and progeny of F1 Lead-toxic rats. Fertile Steril 1971; 22: 755-760.

4. Donldson W. E, Knowles S.O. Is lead toxicosis a reflection of altered fatty acid composition of membranes? Comp. Biochem Physiol 1993; 104: 377-379.

5. Kidd P. Glutathione, systemic protectant against oxidative and free radical damage. Altern Med Rev 1997; 2: 155-176.

6. Pedersen T, Peters H. Proposal for a classification of oocytes and follicles in the mouse ovary. J Reprod Fert 1968; 17: 555-557.

7. Hilderbrand DC, Der RD, Griffin WT, Fahim MS. Effect of Lead acetate on reproduction. Am J Obstet Gynecol 1973; 10: 58-65.

8. Junaid M, Chowdhuri DK, Narayan R. Lead-induced changes in ovarian follicular development and maturation in mice. J Toxicol Environ Health 1997; 50: 31-40.

9. Vermande Van Eck GJ, Meigs JW. Changes in the ovary of Rhesus monkey after chronic lead intoxication. Fertil Steril 1960; 11: 223-234.

10. Stowe HD, Moore RA. The reproductive ability and progeny of F1 Lead-toxic rats. Fertil Steril 1971; 22: 755-760.

11. Thibault C. Experimental approaches to normal and abnormal mammalian oocyte maturation and ageing. In: Boue A, Thibault C, eds. Chromosomal errors in relation to reproductive failure. Paris: Colloque INSERM 1974.

12. Gougeon A, Testart J. Germinal vesicle breakdown in oocytes of human atretic follicles during the menstrual cycle. J Reprod Fertil 1986; 78: 389-401.

13. Kidd P. Glutathione, systemic protectant against oxidative and free radical damage. Altern Med Rev 1997; 2: 155-176.

14. Ercal N, Terese C, Lutz P. L-cysteine protects Chinese hamster ovary (CHO) cells from lead induced oxidative stress. J Toxicology 1996; 108: 57-64.

15. Benoff S, Jacob A, Hurley IR. Male infertility and environmental exposure to Lead and cadmium. Hum Reprod 2000; 6: 107-121.

16. Moldeus P. L-cysteine. Methods in Enzymology 1994; 234: 482-492.

17. Kagi R. J. J, Schaffer A. Biochemistry of metallothioneins. Biochemistry 1988; 27: 8509-8515.

18. Valle B, Falchuk K. The biochemical basis of zinc physiology. Phys Res 1999; 73: 79-118.

19. Cherian M. G, Howell S. B. Nuclear localization of metallothionein during cell proliferation and differentiation, A review. Cell Mol Bio 2000; 46: 347-356.