FUNCTIONAL CHANGES OF TESTES IN LEAD INTOXICATED RATS

A. ROY CHOWDHURY, R. V. RAO, A. K. GAUTAM
and S. K. KASHYAP

*National Institute of Occupational Health,
Meghani Nagar, Ahmedabad 380016, India.*

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Abstract: Lead acetate treatment intraperitoneally at the dosages of 1 mg, 2 mg, 4 mg and 6 mg/kg over a period of 30 days altered spermatogenesis in rats. Inhibition of Δ^5 -3 β -hydroxysteroid dehydrogenase was parallel with the high concentration of testicular cholesterol and low concentration of ascorbic acid, alkaline phosphatase and protein in most of the treated groups. Gradual increase in blood and testis lead alongwith the proportional inhibition of δ -aminolevulinic acid dehydratase levels indicated the intensity of lead toxicity in rats. Data revealed that alteration of spermatogenesis in lead treated rats might be due to the inhibition of steroidogenesis.

Key words: Lead acetate—Spermatogenesis—Steroidogenesis testes—Leydig cells

INTRODUCTION

Reproductive abnormalities in experimental animals treated with lead acetate have already been documented both in males and females.^{1,2)} Sperm motility was impaired in semen samples from rats with blood lead concentrations exceeding 39 μ g/100 ml.³⁾ Moreover, rats, guinea pigs and rabbits showed the impairment of testicular function after treatment with lead acetate.⁴⁾ Little information is available whether these observations are due to direct effect of lead on spermatogenesis or through inhibition of steroidogenesis. Present investigation was undertaken to contribute a better understanding of the testicular function with different dosages of lead acetate treatment in rats.

MATERIALS AND METHODS

Experimental design: Ninety male albino rats of Charles Foster Strain, weighing 150 ± 5 g were obtained from National Institute of Occupational Health breeding colony. Animals were divided into five equal groups (18 rats/group) and maintained under uniform experimental conditions. Group I served as control, while Group II, III, IV and V received aqueous solution of lead acetate daily

intraperitoneally (i.p.) at the dosage of 1 mg, 2 mg, 4 mg and 6 mg/kg body weight respectively over a period of 30 days. Control animals received distilled water as per the experimental schedule. Initial and final body weight of control as well as experimental groups were recorded.

Biochemical estimations: On 31st day, prior to sacrifice, the animals were anaesthetised by solvent ether and blood was collected from retro-orbital venous plexus in heparinized vials for the estimation of blood lead by Atomic Absorption Spectrophotometry.⁵⁾ 0.2 ml of blood was used for the estimation of δ -amino-levulinic acid dehydratase (ALA-D).⁶⁾

After decapitation of animals, testes were cleanly dissected out and weighed. Five testes from each group were collected and separately digested with concentrated nitric acid for the determination of testicular lead by Atomic Absorption Spectrophotometer.⁷⁾ Fifteen testes from each group were homogenized separately in ice cold distilled water and the clean supernatant was obtained after centrifugation. From this supernatant the testicular ascorbic acid, protein and alkaline phosphatase (ALK.Pase) were estimated by the methods of Roe and Kuether,⁸⁾ Lowry *et al.*⁹⁾ and Bodansky,¹⁰⁾ respectively.

Five testes from each group were homogenized in chloroform ethanol mixture (2:1) and the testicular cholesterol was estimated according to Sperry and Webb.¹¹⁾

Histological and histometrical study: Six testes from each group were fixed in 10% neutral formalin, embedded in paraffin and 5 μ m thick sections were stained with periodic acid Schiff's (PAS) and hematoxylin for the microscopical examination. VIIth stage of spermatogenesis was assessed quantitatively due to clear microscopical view of acrosomes in spermarids.¹²⁾ 8.5 Sertoli cells per tubular cross-section were considered as standard. Seminiferous tubular diameter was measured by ocular micrometer at 160 $\times$ magnification. Leydig cell nuclear diameter was determined at 640 $\times$ magnification.¹³⁾

Histochemical study: Fresh frozen 8 μ m thick cryostatic sections of testes were mounted on coverslips and processed to note the activity of Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -OHSD).¹⁴⁾ Control sections were incubated in substrate free media.

Statistical analysis: Statistical significance was determined by Student's t-test.

RESULTS

Increasing doses of lead acetate produced a gradual decrease in body as well as in testicular weight in all experimental groups (Table 1). Blood and testicular

Table 1. Effect of lead acetate on body weight, testicular weight and histometry of testicular tissues

Group	Body weight (g) ^a		Testis weight (g) ^b		Seminiferous tubular diameter ^c (μ m)	Spermatogenic count at stage VII ^c /8.5 Sertoli cell [†]			Leydig cell nuclear diameter ^c (μ m)
	Initial	Final	Absolute	Relative		Spg	R-Spcyt	Pachyt	7-Sptd
I (Control)	150 $\pm$ 3.5	240 $\pm$ 4.5	1.24 $\pm$ 0.09	0.59 $\pm$ 0.03	267.0 $\pm$ 3.55	2.62 $\pm$ 0.11	3.56 $\pm$ 0.15	3.88 $\pm$ 0.16	16.80 $\pm$ 0.52
II (1 mg/kg)	154 $\pm$ 2.8	231 $\pm$ 1.8*	1.10 $\pm$ 0.12*	0.50 $\pm$ 0.04*	263.0 $\pm$ 3.80	2.52 $\pm$ 0.10	3.53 $\pm$ 0.10	3.61 $\pm$ 0.12	13.89 $\pm$ 1.82*
III (2 mg/kg)	154 $\pm$ 1.8	220 $\pm$ 3.1**	1.12 $\pm$ 0.04*	0.41 $\pm$ 0.03**	263.0 $\pm$ 4.40	2.41 $\pm$ 0.08	3.38 $\pm$ 0.11	2.91 $\pm$ 0.10**	12.01 $\pm$ 0.47**
IV (4 mg/kg)	152 $\pm$ 1.2	201 $\pm$ 7.03**	0.73 $\pm$ 0.03**	0.40 $\pm$ 0.02**	265.5 $\pm$ 3.45	1.70 $\pm$ 0.11**	2.21 $\pm$ 0.10**	2.20 $\pm$ 0.12**	10.08 $\pm$ 0.33**
V (6 mg/kg)	150 $\pm$ 1.5	171 $\pm$ 1.8**	0.68 $\pm$ 0.04**	0.37 $\pm$ 0.03**	220.0 $\pm$ 3.16**	1.23 $\pm$ 0.10**	1.64 $\pm$ 0.08**	2.07 $\pm$ 0.11**	9.12 $\pm$ 0.24**
									3.1 $\pm$ 0.13**

Each figure represents mean $\pm$ SE of a: 18 rats; b: 36 testes; c: 20 observations, *P<0.05, **P<0.01, Spg: Spermatogonia, R-Spcyt: Resting spermatocytes, Pachyt: Pachytene spermatocytes, 7-Sptd: spermatids of VIIth stage, †: 8.5 Sertoli cells were considered as standard per seminiferous tubular section.

lead exhibited a significant increase and inhibition of ALA-D was observed in treated animals (Table 2).

Increase in testicular cholesterol and decrease in ascorbic acid, ALK.Pase and protein were observed in Group III, IV and V (Table 2).

In Group II, significant decline of spermatids was noted and there was no alteration of other spermatogenic cells as compared to control (Fig. 1a; Table 1). Inhibition of post meiotic spermatogenic cells namely; pachytene spermatocytes and spermatids was observed in Group III (Fig. 1b; Table 1). Spermatogenic cell count was significantly decreased in Group IV and V (Table 1). Germinal cell layer detachment from basal membrane and injury of spermatocytes and spermatids were exhibited in Group IV (Fig. 1c). Significant degenerative changes and interstitial oedema were observed alongwith atrophy of Leydig cells in Group V (Fig. 1d, e). Moreover, the seminiferous tubular diameter and Leydig cell nuclear diameter were also decreased significantly in Group V (Table 1).

Gradual decrease of testicular Δ^5 -3 β -OHSD activity was noted in all experimental groups as compared to control (Fig. 1f, g, h, i).

DISCUSSION

The retardation of body and testicular weight of rats after lead treatment may possibly be due to relative intensities of lead toxicity which was determined by gradual increase in blood and testis lead and correspondingly the inhibition of blood ALA-D.¹⁵⁾ Administration of lead in rats and rabbits caused marked

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- Figure 1.** a: Section of normal rat testis showing arrangement of spermatogenic cells in the seminiferous tubules and Leydig cells ($\times 160$).
b: Post meiotic spermatogenic inhibition in rat testis treated with 2mg/kg (i. p.) body weight lead acetate over a period of 30 days ($\times 160$).
c: Inhibition of spermatogenesis and detachment of germinal cell layers in the testis of rat received 4 mg/kg (i. p.) body weight lead acetate ($\times 160$).
d: Marked degenerative changes in spermatogenic cells and atrophy of Leydig cells in rat treated with 6 mg/kg (i. p.) lead acetate ($\times 160$).
e: Marked depletion of the spermatogenic cells after 6 mg/kg lead acetate treatment for 30 days ($\times 640$).
f: Showing normal activity of Δ^5 -3 β -OHSD in testicular tissue of control rat ($\times 160$).
g: Rats treated with 2 mg/kg lead acetate (i. p.) daily for 30 days showing slight depletion of Δ^5 -3 β -OHSD activity in the testicular tissue ($\times 160$).
h: Administration of 4 mg/kg lead acetate (i. p.) daily for 30 days, showing less Δ^5 -3 β -OHSD activity in the testis of rat ($\times 160$).
i: Significant inhibition of Δ^5 -3 β -OHSD activity in the testicular tissue after treatment with 6 mg/kg (i. p.) lead acetate daily over a period of 30 days ($\times 160$).

Table 2. Blood and testis lead content, Blood ALA-D and alteration of different biochemical constituents in the testicular tissues of rats after treatment with lead acetate

Group	Blood-Pb	Testis-Pb	Blood ALA-D	Testicular Cholesterol	Testicular Ascorbic acid	Testicular ALK. Pase	Testicular Protein
	($\mu\text{g}/100\text{ ml}$)	($\mu\text{g}/\text{g}$)	($\mu\text{mol}/\text{PBG}/\text{hr}/\text{lit-RBC}$)	($\text{mg}/100\text{g}$)	($\text{mg}/100\text{g}$)	(BU) $\dagger$	(mg/g)
I	5.09 $\pm$ 0.21	0.40 $\pm$ 0.02	226.40 $\pm$ 14.52	331.10 $\pm$ 9.14	55.60 $\pm$ 2.15	535.62 $\pm$ 8.32	82.56 $\pm$ 3.24
II	56.10 $\pm$ 1.45**	2.02 $\pm$ 0.11**	154.61 $\pm$ 9.73**	336.90 $\pm$ 9.03	55.00 $\pm$ 1.80	534.25 $\pm$ 5.50	80.64 $\pm$ 2.34
III	91.51 $\pm$ 2.40**	1.67 $\pm$ 0.10**	139.73 $\pm$ 9.37**	388.64 $\pm$ 16.70**	43.70 $\pm$ 2.20**	269.76 $\pm$ 8.21**	69.62 $\pm$ 3.26**
IV	196.30 $\pm$ 11.00**	2.61 $\pm$ 0.13**	108.00 $\pm$ 12.06**	503.61 $\pm$ 11.40**	21.71 $\pm$ 1.15**	260.08 $\pm$ 1.78**	48.32 $\pm$ 2.81**
V	332.72 $\pm$ 28.53**	4.37 $\pm$ 0.17**	93.12 $\pm$ 16.40**	874.15 $\pm$ 23.38**	20.80 $\pm$ 1.50**	259.38 $\pm$ 21.85**	42.26 $\pm$ 2.59**

Each figure represents mean $\pm$ SE of five samples, ** $P < 0.01$, $\dagger$ Bodansky Units.

decrease in testicular weight and degeneration of spermatogenic cells.¹⁶⁾ Equivocal observations in Group IV and V may possibly be due to significant decrease of testicular protein. Prolonged administration of lead to rats caused significant decrease in liver and kidney protein content.¹⁷⁾ Relative deposition of lead in testicular tissues caused significant decrease in protein content alongwith cellular degeneration in Group III, IV and V. The ALK.Pase in the testes is mainly associated with the structural integrity of the basement membrane of the seminiferous tubules.¹⁸⁾ The inhibition of testicular ALK.Pase in Group III, IV and V were associated with the regression of basement membrane of the seminiferous tubules which was confirmed by histological findings.

The testosterone is synthesized from cholesterol in Leydig cells.¹⁹⁾ Leydig cell degeneration and significant increase in testicular cholesterol in treated groups suggest the failure of testosterone synthesis. A clinical study revealed that serum testosterone levels were significantly reduced in lead exposed men.²⁰⁾ Ascorbic acid plays an important role in the process of steroidogenesis²¹⁾ and Δ^5 -3 β -OHSD is key enzyme for the same process.²²⁾ Significant fall of ascorbic acid and gradual inhibition of Δ^5 -3 β -OHSD alongwith accumulation of cholesterol suggest the inhibition of steroidogenesis in the testes of lead exposed rats.

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