

Protection of adrenal and male gonadal functions by androgen in lead-treated rats

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Abstract

The potential health hazard of the large amounts of Lead that occurs in canned baby food, domestic water from lead-lined tanks and, in printing and petroleum industries.

Lead acetate administration at a dose of 8 mg/kg body weight for 21 days resulted a significant increase in adrenal steroidogenic enzyme (Δ^5 - 3β -HSD) and serum levels of corticosterone, while serum levels of testosterone, FSH, LH and testicular spermatogenesis were decreased in albino rats. But lead-treated rats received exogenous testosterone for the last 14 days of lead treatment, showed prevention of adrenocortical hyperactivity by decreasing adrenal Δ^5 - 3β -HSD activity and serum level of corticosterone. Testosterone administration also increased serum level of testosterone, FSH and LH along with spermatogenesis.

The results suggest that testosterone supplementation in lead-treated rats protects adrenocortical activity and testicular spermatogenesis.

Lead has been an important metal in human society over many years. It occurs in earth crust. Atmospheric lead in cities mostly comes from the exhaust fumes of cars. Many domestic water supplied from lead-lined tank and water pipes contains high concentration of lead. A large amounts of lead that causes a potential health hazard occurs in canned baby food¹. Chronic lead poisoning is often seen in young children from sucking lead toys and in workers engaged in printing and petroleum industries.

Prolonged exposure to lead results inhibitory effects on male reproduction^{2,3}. Serum testosterone level and accessory sex organ weight were also decreased following lead acetate treatment in adult rats⁴. Earlier studies by Ghosh (1995)⁵ have shown that chronic administration of lead acetate develops adrenocortical hyperactivity in rats. Though there are some reports on adverse effects of lead exposure on different endocrine and reproductive organs till today there is no literary data available regarding the prevention of adrenocortical hyperactivity and male gonadal inhibition in lead treated rats by supplementation of male sex hormone.

Therefore, the present study has been undertaken to investigate the effect of testosterone on adrenal steroidogenic enzyme and serum levels of corticosterone, gonadotropins, testosterone and testicular spermatogenesis in lead-treated rats.

Materials and methods

The experiments were carried out on 24 mature albino rats of Wistar strain (BW 150-160 gms). The animals were divided equally into three groups. All the animals were maintained under standard laboratory conditions at 28±2°C with standard rat diet and water *ad libitum*.

Lead acetate received from E. Mark Chemical Co., Bombay, India. 16 animals of two groups were injected intraperitoneally with lead acetate at a dose of 8.0 mg (1.0 ml distilled water), kg⁻¹ BW day⁻¹ for 21 days. Eight animals of one group received 1 ml sterile distilled water kg⁻¹ BW day⁻¹ for the same duration as lead-injected rats. One group of lead acetate treated rats also received testosterone at a dose of 10 mg/0.1 ml of 10% ethanol /100 gm BW day⁻¹ for the last 14 days. All animals were sacrificed after 21 days by cervical dislocation under ether anaesthesia, following protocols and ethical procedure.

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Blood was drawn from the abdominal vein, centrifuged and serum was stored at -20°C until testosterone, corticosterone and gonadotropins were assayed. The testes were dissected out, fixed in Bouin's fluid and embedded in paraffin. Adrenals were removed dissected free of surrounding connective tissue, weight and kept at -20°C for studying the activities of Δ^5 -3 β -hydroxysteroid dehydrogenase. The accessory sex organs were dissected out, trimmed off attached tissues and weighed. The adrenal Δ^5 -3 β -HSD activities were measured following the method of Talalay⁶ and subsequently modified by Biswas et al (2001;2004)^{7,8}. Serum corticosterone was determined by spectroflurometry according to the methods of Glick⁹ and Silber¹⁰. The fluorescence was measured at 463 nm (excitation) and 518 nm (emission) by setting the instrument at a spectroflurometric reading 80 with a standard corticosterone (Sigma Chemical Co., St. Louis, MO, USA) solution having concentration 1.6mg/ml.

Radioimmunoassay (RIA) of hormones

Serum FSH and LH were measured by RIA as described in the instructions provided with the Kits (NIADDK USA). Pure rat FSH (NIDDK-rFSH⁻¹⁻⁵) were iodinated using chloramines T (Sigma Chemical Co., USA) method of Greenwood et al (1963)¹¹. Goat anti rabbit γ -globulin was used as the second antibody. Serum samples were expressed as μ g/L of serum. The intra assay variations were 6% and 5% for FSH and LH respectively.

RIA of serum testosterone was carried out using testosterone ¹²⁵I RIA Kit (ICN Biochemical Inc., Diagnostic Division, Costa Mesa, CA 92626, USA). Radioactivity was determined by the Gamma scintillation counter at Bose institute Calcutta, India. All samples were run in duplicate in a single assay to

avoid interassay variations. The interassay coefficient of variations for testosterone was 6.5%. Since chromatographic purification of the sample was not performed, each testosterone value was the sum of testosterone and dihydrotestosterone.

Study of Spermatogenesis

Paraffin-wax sections (5 μ m thick) were taken from mid portion of testis and stained with periodic-acid Schiff-haematoxylin. The relative number of each variety of germ cell at stage VII of the seminiferous epithelium cycle, i.e. type-A spermatogonia (ASg), preleptotene spermatocytes (pLSc), mid-pachytene spermatocytes (mPSc) and step-7 spermatids (7Sd) were counted according to the method of Leblond and Clermont (1952)¹². The different nuclei of germ cells were counted in 20-22 round tubular cross-sections at stage VII in each rat testis. All the nuclear counts of the germ cells were corrected for differences in nuclear diameter by the formula of Abercrombie (1946)¹³, i.e. true count =(crude count xsection thickness)/(section thickness + nuclear diameter of germ cells) and tubular shrinkage by Sertoli cell correction factor (Clermont & Morgentaler 1955)¹⁴.

Results

There was a significant increase in adrenal weight, adrenal Δ^5 -3 β -HSD activity and serum level of corticosterone in rats treated with lead acetate in comparison with control rats (Table 1). But the lead treated rats showed a significant fall of all the parameters towards normal level after Testosterone administration. Accessory sex organs weights were decreased after lead treatment, but supplementation of testosterone caused significant recovery of accessory sex organs weights.

Table 1: Changes in adrenal weight, adrenal Δ^5 -3 β -HSD activity, serum level of corticosterone and prostatic and seminal vesicular weight after lead administration for 21 days and supplementation of testosterone for the last 14 days. Values are mean SEM (n=8)

Group	Adrenal (pair) (mg/100 gm body wt)	Δ^5 -3 β -HSD (unit/mg tissue per h)	Serum level of corticosterone (mg/100ml)	Prostate Weight (mg %)	Seminal vesicle weight (mg %)
Control	1.367 \pm 1.27 ^a	20.79 \pm 1.47 ^a	35.05 \pm 1.09 ^a	190.46 \pm 12.76 ^a	382.64 \pm 15.50 ^a
Lead acetate	22.05 \pm 0.78 ^b	28.17 \pm 1.30 ^b	47.35 \pm 1.39 ^b	158.82 \pm 11.77 ^b	160.08 \pm 14.27 ^b
Lead acetate and Testosterone	15.25 \pm 1.15 ^a	22.98 \pm 1.04 ^a	39.45 \pm 1.67 ^c	181.56 \pm 12.68 ^a	345.36 \pm 14.55 ^c

Result obtained were compared by ANOVA and multiple comparison of two tailed t-test at $p < 0.05$; in any vertical column, the means with same superscript do not differ from each other significantly.

Table 2: Effect of Lead acetate and testosterone on number of germ cells per tubular cross section at stage VII of the seminiferous epithelial cycle in rats (mean±SEM.n=8)

Group	ASg	pLSc	mPSc	7Sd	mPSc:7Sd	7Sd degeneration %	Effective 7Sd degeneration
Control	0.63±0.02 ^a	18.11±0.51 ^a	19.50±0.60 ^a	62.58±1.22 ^a	1:3.20	19.76 ^a	
Lead Acetate	0.40±0.30 ^b	12.88±0.63 ^b	12.82±0.83 ^b	34.72±1.51 ^b	1:2.70	32.56 ^b	12.80
Lead Acetate And Testosterone	0.50±0.02 ^c	12.60±0.50 ^b	13.48±0.61 ^b	43.18±2.10 ^c	1:3.20	19.92 ^a	0.16

Spermatogonia A; pLSc=preleptotene spermatocyte; mPSc=mid pachytene spermatocyte; 7Sd= step 7 spermatid. In any vertical column, the means with different superscripts differ significantly (p<0.05) from each other (analysis of variance and multiple comparison two-tailed t test).

Quantitative analysis of the testicular germ cells at stage VII of seminiferous tubule revealed that lead treatment significantly reduced the number of ASg, 7Sd, mPSc, and mature spermatozoa (Table 2). Significant recovery of ASg and 7Sd were noted after administration of testosterone when compared with the control. The percentage of spermatid degeneration was 32.56% after lead treatment. Spermatid degeneration was reduced to normal level after testosterone administration to lead treated rats. The effective 7Sd degeneration was also significant in lead treated rats but reduced markedly after testosterone supplementation. Serum levels of testosterone, FSH, LH were decreased significantly after lead administration when compared with controls but supplementation of testosterone to the lead treated rats showed a significant rise of all the hormones towards normal level (Fig 1)

Discussion

This study has demonstrated that administration of lead acetate in rats, there is a significant increase in adrenal steroidogenic enzyme, Δ^5 -3 β -HSD activity and serum level of corticosterone but fall of serum testosterone and gonadotropins along with inhibition of spermatogenesis. The steroidogenic enzyme, Δ^5 -3 β -HSD is directly involved in cortical hormone synthesis. Therefore, stimulated Δ^5 -3 β -HSD activity increases synthesis of corticosterone. But administration of testosterone decreases Δ^5 -3 β -HSD activity and serum level of corticosterone, similar inhibition of adrenocortical activity has been observed in anti GnRh treated rats when serum level of testosterone is increased. Testosterone possibly inhibits corticosterone synthesis by decreasing ACTH secretion in lead-treated rats.

The quantitative analysis of the epithelium at stage VII of the seminiferous cycle reveals that lead treatment reduces the number of ASg, mPSc and 7Sd significantly. The reduction in the number of ASg in lead treated rats is possibly due to increased rate of degeneration of ASg.

Theoretically the pachytene spermatocyte : spermatid ratio should be 1:4 (Cleromnt and Morgentaler, 1955¹⁴) but 1:3.20 ratio in our control rats indicates 19.76 % spermatid degeneration. This ratio becomes 1:2.70 in lead treated rats, indicating that during the process of spermatocyte to spermatid conversion 32.56% of the cells are degenerated. Administration of testosterone to lead treated rats shows similar degeneration as observed in control rats. Since testicular germ cell development up to the level of step 7 spermatids and meiotic division of primary spermatocytes to spermatids¹⁸ are supported by FSH. A reduction in the number of spermatogonia, spermatocytes and spermatids in lead treated rats may be due to low serum levels of FSH. On the other hand several studies in male rats have shown that cortisol inhibits the release of LH after Orchidectomy^{19,20}. Thus a decrease in serum corticosterone towards the normal level after administration of testosterone in lead treated rats removes the inhibitory effect of corticosterone in LH secretion which results an increased LH secretion.

In conclusion our findings indicate that chronic administration of lead to albino rats results adrenal hyperactivity along with inhibition of spermatogenesis but lead treated rats after supplementation with testosterone shows almost normal adrenocortical activity and spermatogenesis.

Acknowledgement

The authors would like to thank Prof. Veena Bapat, Head of the Department of Physiology, Kathmandu Medical College, Nepal for encouragements for this work. Thanks are also to Prof. A.K. Chatterjee, Head of the Department of Physiology, Calcutta University, Calcutta for his co-operation and infrastructural support.

Reference

1. Mitchell RL, aldous KM. Mass screening for lead poisoning. *Environ Heath Prospect Exp Issue*. No 7, 1979;P59.
2. Chowdhury AR, Rao RV, Gautan Ak. Histochemical changes in the testis of lead-induced experimental rats. *Folia Histochem cytobiol* (1986;24:233-237. cytohid,
3. Sokol pz, Madding CE, Swerdloff PS. Lead toxicity nad the hypothalamic pituitary testicular axis. *Biol Reprod*, 1985; 33:722- 728
4. Biswas NM, Ghosh P. Effect of lead on male gonadal activity in Albino Rats. *KUMJ*, 2004; 2:35-42.
5. Ghosh Pk. Effect of lead acetate on testicular and adrenocortical activities in albino rats, Thesis, Calcutta University, India 1995.
6. Talalay P. Hydroxy steroid dehydrogenase. in: Colowick, Kaplan eds. *Methods in enzymology*. New York: Academic press, 1962;5:512-516
7. Biswas NM, Chaudhari GR, et al. Effect of easein diet on gonadotropin relasing hormone antagonist induced changes in adrenal gonadal functions in male ras. *Indian J Exp Biol*. 2001; 39: 1249-1253.
8. Biswas NM, Chattopadhyay A, Sakar M. Effect of gold on testicular steroidogenic and gametogenic functions in immature male albino rats. *Life Sci*. 2004;75(6):629-636.
9. Glick D, Redlich DV, Lavine S. Fluorometric determination of corticostrone and cortisol in 0.02-0.05 milliliters of submilligram sample of adrenal tissue. *Endocrinology*, 1964; 74:653
10. Silber Rh, in: Glick ed. *Method of biochemical analysis*. Interscience publishers, New York, 1966;63.
11. Greenwood FC, Hunter WM, Glover JS. The preparation of ¹³¹I labeled human growth hormone of high specific activities. *Biochem J*. 1963; 89: 114-123.
12. Leblond PC Clermont Y. Definition of the stages of the semimiferous epithelium in the rat. *Ann N Y Acad Sci*. 1952; 55: 548-573.
13. Abercrombie M. Estimatiin of nuclear population from microtome sections. *Anat Rec*. 1946; 94 :239-247.
14. Clermont Y, Morgentalor H. Quantitative study of spermatogenesis in hypophysectomized rats. *Endocrinology* 1955;57:369-382.
15. Biswas NM, Sengupta R, et al. Prevention of adrenocortical hyperactivity by dietary casein in rats exposed to forced swimming stress. *Indian J Exp Biol*. 2001;39:178-180
16. Bartlett JMS, Weinbauer GF, Nieschlag E. Differential effects of FSH and testosterone on the maintenance of spermatogenesis in the adult hypophysectomized rat. *J Endocrinol*. 1989;121:49-58.
17. Vaishnav MY, Moudgal RN. folliclestimulating horrnone regulation of seeretion and molecular mechanism of action, in Ringstrom and Schwartz eds. *Spring verlag*, New York 1992;364-368.
18. Hsuch AJ, Jones BC, Gonadotropin releasing hormone: Extrapituitary actions and paracrine control mechanism, *Ann Rev Physiol*. 1983;45:83
19. Ringstrom SJ, Schwartz NB. Examination of prolactin and pituitary adrenal axis components as intervening variables in the adrenalectomy induced inhibition of gonadotropin response to castration. *Endocrinology*. 1984;114:880