CHANGES CAUSED BY FLUORIDE AND LEAD IN ENERGY METABOLIC ENZYME ACTIVITIES IN THE REPRODUCTIVE SYSTEM OF MALE OFFSPRING RATS

Haitao Liu,[ab] Ruiyan Niu, [a] Jinming Wang[a], Ying He, [c] Jundong Wang [a]

Shanxi, China

SUMMARY: To assess effects of fluoride (F) and lead (Pb) on the energy metabolism of the male reproductive system, the activities of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), adenosine triphosphatase (ATPase), and Gamma-glutamyltranspeptidase (γ-GT), along with sperm quality and testicular histology, were determined at week 6, 8, 10, and 12 in male offspring rat pups exposed in their drinking water either to sodium fluoride (150 mg/L=HiF) or to lead acetate (300 mg/L =HiPb). Compared with the control, LDH activities were significantly increased, whereas SDH activities were decreased in the HiF and HiPb group. Both F and Pb resulted in lower ATPase activity. Additionally, changes in γ-GT activities were also observed, which were decreased in the HiF group and increased in the HiPb group. In contrast to the control group, the F-treated and Pb-treated rats exhibited a marked decline in sperm density and sperm viability along with a significant increase of sperm abnormalities over the entire 12-week study period. Moreover, F and Pb obviously affected the testicular histology, finally resulting in significant increases in the diameter and thickness of seminiferous tubules. Therefore, F and Pb may share a similar reproductive toxic mechanism by which disordered energy metabolism in the testis and epididymis influenced the sperm quality.

Keywords: Energy metabolism enzymes; High fluoride; High lead; Male offspring rats; Sperm quality.

INTRODUCTION

Accumulated data suggest there is a close relationship between declining reproductive health and environmental pollutants like fluoride (F) and lead (Pb).[1-6] Reproductive dysfunction induced by F has distinct morphological and biochemical features such as disorganized germinal epithelia, giant cells in the lumen, decreased sperm quality, and low androgen levels.[7,8] Meanwhile, earlier investigations indicate that high lead exposure can also reduce sperm quality, decrease sperm count and motility, and alter sperm morphology.[9,10] Although the pathway involved in producing infertility appears to vary, previous studies suggest that disturbance of energy metabolism plays an important role in reducing sperm activity and blocking sperm maturation.[11, 12] Moreover, in recent years, with increasing numbers of reports indicating low sperm quality caused by F and Pb alone, epidemiological investigations indicate that F pollution is accompanied by enhanced Pb levels in drinking water.[13, 14] The present experimental study was carried out, therefore, with the aim of studying sperm quality and the activity of certain enzymes involved in energy metabolism in the testis and epididymis of male offspring rat exposed to elevated levels of F and Pb.

[a]For Correspondence: Prof Jundong Wang, Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801, PR of China; E-mail: wangjd@sxau.edu.cn [b]Shanxi Zhengda limited company, Taiyuan, 030006, PR of China. [c]Tianjin Tinashili Limited Company, Tianjin, 300000, PR of China.

MATERIALS AND METHODS

Animals: Forty-eight adult Wistar albino rats (8 weeks old, with a mean body weight of 160±9.4 g), were obtained, together with their standard diets, from the Experimental Animal Center of Shanxi Medical University. The rats were maintained on these normal diets under standard temperature (22–25°C), 12/12-hr light/dark cycle, ventilation, and hygienic conditions.

Establishment of animal model: One male and two females were kept per cage for mating. After the vaginal plug appeared, all female animals were separated and placed in separate cages. These females were divided into control and two experimental groups for treatment purposes as follows: (1) Control group with 10 female rats: received F-free distilled water; (2) High fluoride (HiF) group with 11 female rats: received NaF (150 mg/L) on the day of delivery (day 0) up to day 21; (3) High lead (HiPb) group with 11 female rats: received lead acetate (300 mg/L) on the day of delivery (day 0) up to 21 day. Then the male offspring rats (8 to 12 pups per litter) were separated and given the same levels of NaF and lead acetate as the adult rats from weaning to the age of week 6, 8, 10, and 12.

Haematoxylin and eosin (HE) staining: After cervical dislocation of six male offspring rats in each group at week 6, 8, 10, and 12, the testes were quickly removed and rinsed with distilled water to remove blood. Each of the left testicles was cut into two pieces, which were separately immersed in 10% neutral formalin for 16 hr for tissue fixation. Afterward they were again rinsed with distilled water, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. Finally, they were cut into 6-µm sections with a rotary microtome and stained with haematoxylene and eosin.

Evaluation of sperm quality: The left cauda epididymal of sperm suspension was prepared in normal saline at 37ºC. Sperm viability (live/dead ratio) and sperm density were calculated by the method of Prasad et al.[15] and expressed as percentage viability and density as $\times 10^{10}$/L, respectively. The percentage of abnormal sperm was scored in 10 to 20 separate fields using 1% trypan blue by the method of Talbot and Chacon.[16]

Assay of enzymes activities in testis and epididymis: The right testis and epididymis were weighed, and then homogenized with 1:9 (w/v) 0.9% saline solution at 4°C. Total protein content and the activity of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) activity in testis, and the activity of gamma-glutamyltranspeptidase (γ-GT) and adenosine triphosphatase (ATPase) in epididymis tissues were determined with the enzyme reagent kit provided by the Nanjing Jianchen Biological Institute.

Statistical analysis: Data are expressed as mean±SD. Differences in all parameters between control and HiF or HiPb groups were statistically analyzed by Student's t test using SPSS 11.5 software.

RESULTS

Morphology of testis tissue: As shown in Table 1, the diameter of seminiferous tubules was significantly decreased in both the HiF and HiPb groups during the

entire 12 weeks, compared with the controls. Data for diameter in HiPb group was obviously higher than that in the HiF group at weeks 8 and 10. The thickness of seminiferous tubules was significantly increased at the early observation stages, and then decreased in the HiF group as compared with the control. In the HiPb group, the similar trends also occurred. However, no change was observed between the HiF and HiPb groups except at week 12. Histological images in the Figure reveal the structure changes in testis of the male offspring rats after 12 weeks of exposure to HiF and HiPb.

Table 1. Diameter and thickness of seminiferous tubules of male offspring rats treated with HiF or HiPb (mean±SD, n=6)

	Week	Control group	HiF group	HiPb group
Diameter	6	187.2±6.62	162.5±3.37†	138.6±1.25†§
	8	198.0±2.34	155.1±2.47†	175.1±1.24†§
	10	226.9±1.04	162.3±0.92†	211.7±2.48†§
	12	257.9±1.10	245.5±2.00†	220.2±1.32†§
Thickness	6	34.97±0.73	46.55±0.45†	48.61±0.20†
	8	39.68±0.44	45.00±0.58†	44.43±0.08†
	10	43.27±0.24	38.33±0.32†	37.98±0.45†
	12	50.03±0.26	49.49±0.50	44.96±0.06†§

$^{\dagger}p<0.01$ (compared with the control group), $^{\S}p<0.01$ (HiPb Group compared with HiF Group).

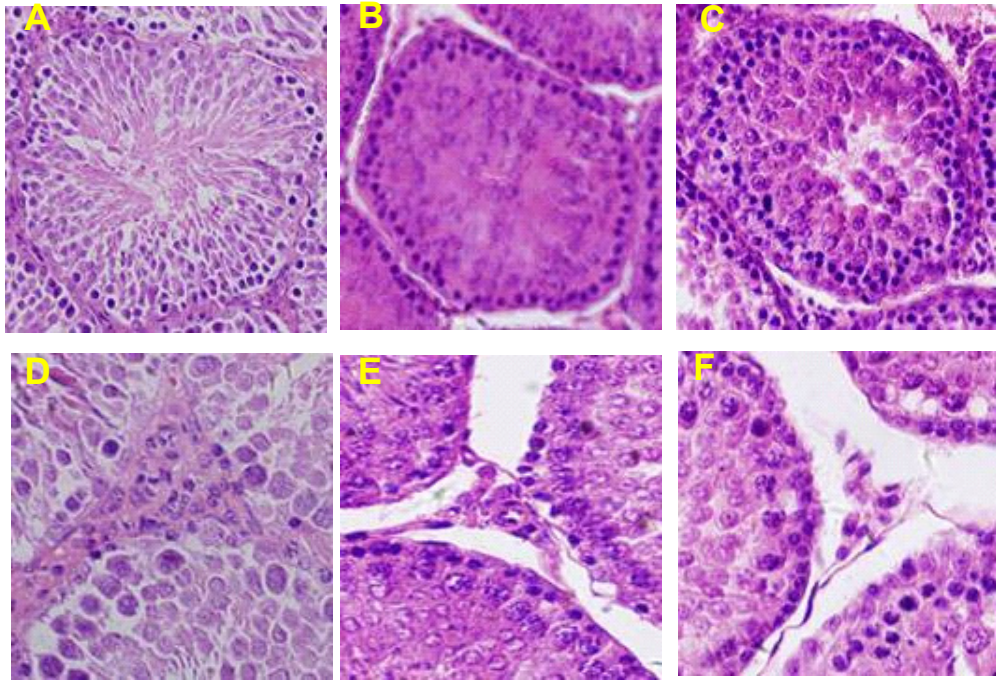

Figure. The photomicrographs show the structure changes in seminiferous tubules (A, B and C, ×330) and Leydig cells (D, E and F, ×660) of male offspring rats at week 12 in the control, HiF, and HiPb groups. The control rat testis shows normal seminiferous tubules (A) and neatly arranged and compact Leydig cells (D). Compared with the control group (A and D), the HiF group (B and E) and the HiPb group (C and F) exhibit disorganized germinal epithelia, and giant cells are observed in the lumen.

Changes in sperm quality: From Table 2, it can be seen that sperm viability and density were significantly increased in the HiF and HiPb rats as compared with the controls, whereas sperm abnormality was significantly increased in both treatment groups. For these three indexes, there is no significant difference between the HiF and HiPb groups except for sperm density and abnormality at week 6.

Table 2. Sperm quality in male offspring rats treated with HiF or Hi Pb (mean±SD, n=6)

	Week	Control group	HiF group	HiPb group
Sperm density (10^6/L)	6	4.75±0.49	3.88±0.54*	2.44±0.68†§
	8	6.18±0.34	5.41±0.41*	5.31±0.44*
	10	9.38±0.36	7.88±0.41†	8.08±0.46†
	12	15.43±0.46	13.67±0.37†	13.9±0.41†
Sperm viability (%)	6	90.69±1.66	72.45±2.78†	69.11±3.58†
	8	91.37±2.64	77.29±3.15†	71.09±4.05†
	10	80.65±3.13	60.74±4.54†	66.49±4.91†
	12	78.81±3.56	54.65±6.01†	58.88±2.89†
Sperm abnormality ratio (%)	6	7.18±0.69	17.46±1.33†	25.64±1.10†§
	8	7.81±0.66	19.86±2.84†	22.41±2.91†
	10	14.64±0.5	29.5±3.15†	25.89±2.26†
	12	18.71±1.16	32.88±3.65†	29.84±3.27†

$^{*}p<0.05$, $^{\dagger}p<0.01$(compared with the control group), $^{\S}p<0.01$(HiPb group compared with HiF group).

Biochemical parameters: Results in Table 3 indicate that administration of NaF for 12 weeks caused no change in LDH activity in testis of the male offspring rats, however, lead caused significant increased at week 8, 10, and 12. Additionally, except that at week 6, data for LDH activity in HiPb group was obviously higher than that in HiF group. Compared with the control, the activities of SDH were markedly decreased in the HiF group, whereas no significant effect was observed in the HiPb group at week 8, 10, and 12.

Table 4 presents the results of the γ-GT and ATPase activities in the epididymis. Compared with the control, the activities of γ-GT were significantly decreased in the HiF and HiPb groups, but there were no differences between these two treatment groups except for the data at week 8. For ATPase activity, it was obviously reduced at week 6, 8, and 10 in the HiF group in comparison with the controls. Rats treated with HiPb also exhibited statistical differences in ATPase activity

Table 3. LDH and SDH activities in testis of male offspring rats treated with HiF or HiPb (mean±SD, n=6)

	Week	Control group	HiF group	HiPb group
LDH activity (U/g protein)	6	2.69±1.41	2.82±1.48	2.15±1.32†§
	8	2.57±1.43	2.54±1.49	2.73±1.49†§
	10	2.78±1.37	2.28±1.45†	3.51±1.95†§
	12	2.62±1.94	2.68±1.94	2.72±0.71†§
SDH activity (U/g protein)	6	2.90±0.09	2.49±0.10†	2.58±0.12†
	8	2.20±0.09	1.87±0.14†	2.58±0.15
	10	2.77±0.13	2.31±0.13†	2.63±0.21
	12	2.97±0.17	2.44±0.18†	2.63±0.22

†p<0.01 (compared with the control group), §p<0.01 (HiPb group compared with HiF group).

Table 4. γ-GT and ATPases activities (U/g protein) in epididymides of male offspring rats treated with F or Pb (mean±SD, n=6)

	Week	Control group	HiF group	HiPb group
γ-GT activity (U/g protein)	6	446.8±35.38	275.1±29.74†	291.7±38.51†
	8	466.7±41.76	289.3±51.97†	460.9±43.56‡
	10	663.9±25.90	619.7±18.70*	600.8±22.00*
	12	889.2±27.10	848.3±24.10*	796.4±18.90*
ATPases activity (U/g protein)	6	1.67±0.04	0.86±0.03*	1.56±0.18§
	8	1.31±0.06	0.78±0.08*	1.83±0.06†§
	10	1.78±0.05	0.49±0.01†	0.54±0.03†
	12	0.91±0.09	1.10±0.17	1.65±0.37*

*p<0.05, † p<0.01 (compared with the control group), ‡p<0.05, §p<0.01 (HiPb group compared with HiF group).

DISCUSSION

Effects of F versus Pb on testicular morphology and sperm quality: In the present study, male offspring rats treated with F and Pb for 12 weeks exhibited disordered arrangement of germ cells and Leydig cells, a decreased spermatogenic cell layer in the seminiferous tubules, and giant cells in the lumen. These findings support the results from other reports which indicated that F[17-20] in various dosages and Pb[21] altered testis histology resulting in structural defects in spermatids and sperms in mice, rats, and rabbits. Quantitative analysis showed that in the HiF and HiPb groups the diameter of seminiferous tubules significantly decreased, while the tubule well thickness increased markedly over the entire 12-week period compared with the controls. We therefore postulate that disordered arrangement of spermatogenic cells and a decrease in cell layer may induce the observed changes in diameter and wall thickness, thereby indicating that F and Pb inhibit testis growth.

Since sperm originates from spermatogenic cells, injury to these cells caused by toxicants can be expected to have a direct influence on sperm quality. In this study, administration of F and Pb resulted in an obvious decline in sperm

density and sperm viability and a significant increase of sperm abnormalities, in agreement with other reports.[22-26] Damage caused by Pb, however, appeared to be restricted mainly to the younger offspring rats.

Effects of F versus Pb on energy metabolism: It is well known that energy from metabolism is stored in ATP that is essential for the energy consumption of sperm and the maintenance of sperm membrane structure during the process of spermatogenesis and sperm maturation. Therefore activities of energy metabolism enzymes in the testis and epididymis appear to be worthy of studying.

SDH is an important enzyme in the Krebs cycle involving the transformation of fructose to sorbitol and then to glucose. The reduction in activity of SDH may block the cycle with a probable accumulation of succinate. Chinoy et al. reported declines in SDH activity level in mice treated with NaF for 30 days.[27] Laszczyca found decreased SDH level in rats exposed to high lead.[28] Hence, our results of the low SDH activity in the HiF and HiPb groups were well confirmed by the above reports.

Anaerobic glycolysis, in which process LDH plays a role, provides most of the energy needed in sperm motility.[29] The content and activity of LDH are major sensitive indexes in the investigation of reproductive toxicity. Although it has been reported that F and Pb exposure reduce LDH activity,[28,30-32] there was a significant increase in the activity of this enzyme in the HiPb group but no appreciable change in the HiF group, which is difficult to explain. One can speculate that the high concentration of F damaged the sperm plasma membranes, which may be related to an outflow of the acrosome content.[33-34]

On the other hand, besides the changes of energy enzymes in the testis, it is worth noting effects of F and Pb on enzymes in the epididymis, the site of sperm maturation. ATPase is an essential enzyme in releasing energy in ATP in order to supply energy needs for sperm motility and metabolism. As reported by others,[35-36] the decrease in activities of activity of ATPase after F treatment was observed in our study. In addition, Pb also reduced the ATPase activity; however, the degree of reduction was no more than that induced by F in the present investigation. The activity of γ-GT in epididymis also decreased significantly with F and Pb treatment. γ-GT is the key enzyme in the γ-glutamyl cycle and plays an important role in the absorption, transport, and synthesis of amino acids and proteins. Disturbances of the activity of γ-GT could indicate damage to the epididymis.

Taking all the above together, the reduction of enzyme activity caused by HiF and HiPb in the rat pups has been found to correlate with alterations in testis morphology and sperm quality, including low viability and high abnormality, thereby suggesting that disturbance of energy metabolism may be one of the mechanisms by which F or Pb affects the male reproductive system.

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