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LEAD EFFECTS ON PITUITARY-TESTICULAR AXIS IN *GUINEA PIGS*

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This study analyzes lead effects in gonadotropins, testosterone, estradiol and progesterone secretion. Lead was applied by intraperitoneal doses of 0.0036 mg/kg/day (TDI), 2,61 mg/kg/day (intermediate) and 4,95 mg/kg/day (LD_{50}–5%) to male mature guinea pigs during 60 days. The hormones secretion profile and the lead accumulation were monitored weekly by analyzing the blood samples. Lead exposure does not seem to affect greatly estradiol, progesterone and LH secretion in blood but the correlations in some cases result significantly. Different doses of lead applied show different effects in testosterone secretion. Low dozes increase testosterone concentration in blood (r = 0.78, α = 0.05) while high dozes decrease it (intermediate dose r = –0.83, α = 0.05 and LD_{50} – 5%, r = –0.87, α = 0.05). The secretion of FSH increases quickly with the application of the highest dose (r = 0.93, α = 0.05). The high secretion of FSH while the testosterone secretion decreases is an indicator of Leydig cells failure confirmed by histological examination of testicules.

Key words: lead; estradiol; progesterone; testosterone; LH; FSH

ЕФЕКТИ НА ОЛОВОТО ВРЗ ПИТУИТАРНО-ТЕСТИКУЛАРНАТА ОСКА КАЈ ГВИНЕЈСКИТЕ СВИЊИ

Ова истражување ги анализира ефектите на оловото врз лачењето на гонадотропините, тестостеронот, естрадиолот и прогестеронот. Оловото беше давано со интраперитонеални дози од 0,0036 mg/kg/ден (TDI), 2,61 mg/kg/ден (средно) и 4,95 mg/kg/ден (LD_{50} – 5%) на машки приплодни гвинејски свињи во текот на 60 дена. Профилот на хормонско лачење и акумулацијата на оловото беа надгледувани неделно анализирајќи примероци од крв. Се чини дека изложувањето на олово не влијае многу врз лачењето на естрадиол, прогестерон и лутеинизирачки хормон (LH) во крвта, но корелациите во резултатите на одредени случаи се значајни. Различни дози на применото олово покажуваат различни ефекти во лачењето на тестостеронот. Малите дози ја зголемуваат концентрацијата на тестостеронот во крвта (r = 0,78, α = 0,05), додека високите дози ја намалуваат (средна доза r = –0,83, α = 0,05 и LD_{50} –5%, r = –0,87, α = 0,05). Лачењето на фоликуло стимулирачкиот хормон (FSH) се зголемува брзо со примената на најголемата доза (r = 0,93, α = 0,05). Високото лачење на FSH додека лачењето на тестостеронот се намалува е индикатор за недостигот на Leydig-овите клетки, потврдено со хистолошки испитувања на тестикулите.

Клучни зборови: олово; естрадиол; прогестерон; тестостерон; лутеинизирачки хормон (LH); фоликуло стимулирачки хормон (FSH)

1. INTRODUCTION

Environmental exposure to lead toxic levels, occurs in many industries and has potential adverse effects in reproduction capacity of exposed men. Clinical studies in animals show that, anomalies of spermatogenesis result from toxic lead exposure but pathogenic mechanisms involved are not identified.

Most of the lead absorbed in organism is excreted via renal filtration or biliary purification. After absorption, lead spreads in three main com-

partments: blood, soft tissues and mineralized tissues. Adult bones and teeth contain more than 95% of total body burden of lead. Even though the blood contains only a small part of total body burden, it serves as the initial deposit of absorbed lead and spreads it in organism, making it available to other tissues (or to excretion).

The half-life of lead in adult human organisms is estimated to be 28 to 36 days. BLL (Blood Lead Level) in venous blood is the most useful screening and diagnostic test for late or ongoing exposure to lead. Lead affects mainly the nervous system, renal function, blood cells, D vitamin and calcium metabolism but there are evidences of adverse effects in reproductive system too (FEDRIP, 2005; ATSDR, 2005; Acharya & Mishra, 2003; Veeramachaneni et al, 2001**).**

The purposes of this study are to follow the dynamic of lead accumulation in the blood, estimate the effects of different doses of lead in male guinea pig reproduction, estimate the effect of lead in gonadotropic hormones and sexual steroid hormones secretion in blood circulation.

Taking in consideration that lead occupies the second place in the CERLA priority list of hazardous substances, it is of special importance to evidence lead's adverse effects, as well as in male reproduction processes.

2. MATERIALS AND METHODS

In this study 40 individuals of the specie *Cavia porcellus* were selected. They were all males and sexually matured with a body weight between 400 and 550 g (approximately 4 months old) clean of infective disease. They were divided into 4 groups of work: 0 – control group treated with physiologic water by intraperitoneal injection; 1 – TDI group treated with aqueous solution of lead acetate by intraperitoneal injection (0.0036 mg lead/kgbw/day); 2 – intermediate group treated with aqueous solution of lead acetate by intraperitoneal injection (2.16 mg lead/kgbw/day); 3 – LD_{50} – 5% group treated with aqueous solution of lead acetate by intraperitoneal injection (4.95 mg lead/kgbw/day). The solution of lead acetate was prepared with dejonized and sterilized water, and applied for 60 days consequently. The animals were kept in the same conditions of temperature, lightning and humidity. They were also treated with the same regimen of nutriment.

In the beginning of the experiment and every one week, till the scarification day, blood samples were collected to measure the lead levels. Venous blood collection was made in intracardiac way. Measurements of lead in blood were made in the Atomic Absorption Spectrometer "Varian" type with detections limit 0.06 ppb (μg/l). Measurements of gonadotropic hormones and sexual steroid hormones in serum using ELISA principle were also made. These measurements were made automatically in the Elecsys 2010 system with detections limits: estradiol 5.0 pg/ml; progesterone 0.030 ng/ml; testosterone 0.069 nmol/l; LH 0.10 mIU/ml and FSH 0.10 mIU/ml.

3. RESULTS AND DISCUSSION

Each week, during the treatment period, venous blood samples were taken and sexual steroid hormones (estradiol, progesterone and testosterone) and gonadotropic hormones were measured in the serum. The measured values are transformed by means of working groups (zero values are those under the minimum level of measurement).

The tables and figures below represent the connections between lead accumulation in blood and different hormone secretion at the end of the treatment period referring to the different dozes of lead applied in each group.

In Table 1 the values of lead concentration in blood and estradiol in serum at the end of the treatment (according to the doses applied) are given.

Figure 1 shows the cloud of spots and the linear regression line of this distribution. The linear function that connects these two parameters is expressed by the equation $y = 2.0879x + 17.716$ ($a = 17.716$ and $b = 2.0879$) with the determination coefficient $R^2 = 0.4863$, so this line explains approximately 49% of the real values of the distribution. In order to determine the strength of the connection between BLL and the estradiol level in serum in males treated with lead, Pearson's correlation coefficient was computed and its value resulted $r = 0.697$ for $\alpha = 0.05$ confidence level. So we can say that this is a significant positive corre-

lation and this means that lead induces in males the rise of estradiol secretion in blood.

Table 1

Concentration of lead in blood and estradiol in serum in male Guinea pig

Dose	Concentration	
	Pb (ppm)	Estradiol (pg/ml)
Control	0.000	16.09
TDI	0.147	18.84
Int. dose	1.173	22.48
LD_{50}–5%	1.882	20.14

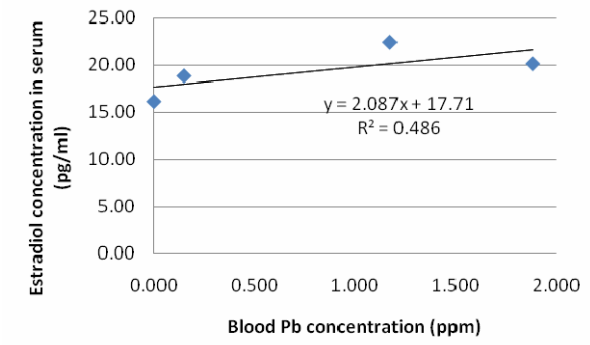

Fig. 1. Statistical connection between Pb in blood and estradiol levels in serum in male *Guinea pig*.

Table 2 and Figure 2 show the values and the distribution of the cloud of spots for lead concentration in blood and progesterone concentration in serum of males treated with lead.

Table 2

Concentration of lead in blood and progesterone in serum in male Guinea pig

Dose	Concentration	
	Pb (ppm)	Progesterone (ng/ml)
Control	0.000	0.132
TDI	0.147	0.137
Int. dose	1.173	0.284
LD_{50}–5%	1.882	0.196

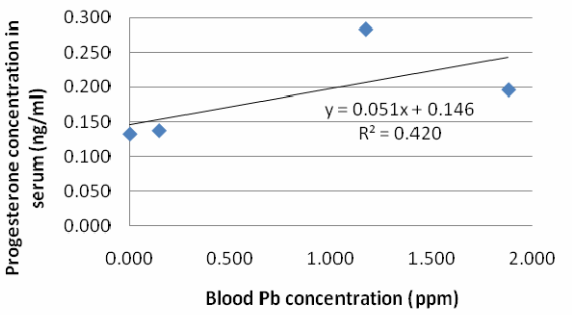

Fig. 2. Statistical connection between BLL and progesterone levels in serum in male *Guinea pig*

Examination of this relationship resulted in the linear regression equation $y = 0.0516x + 0.146$ with the determination coefficient $R^2 = 0.4207$. The strength of this connection is determined by the Pearson's coefficient $r = 0.649$ for $\alpha = 0.05$ level of confidence. As for the estradiol, the connection between BLL and the progesterone secretion in blood is significantly positive but not very strong.

Table 3 shows the BLLs and testosterone levels in serum at the end of the experiment in males treated with lead and grouped according to the doses applied.

Table 3

Concentration of lead in blood and testosterone in serum in male Guinea pig

Dose	Concentration	
	Pb (ppm)	Testosterone (nmol/l)
Control	0.000	7.64
TDI	0.147	27.34
Int. dose	1.173	0.99
LD_{50}–5%	1.882	0.63

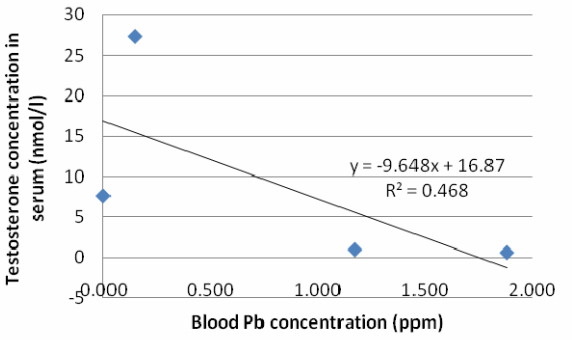

Fig. 3. Statistical connection between BLL and testosterone levels in serum in male *Guinea pig*

Figure 3 illustrates the distribution of the cloud of spots and the linear regression line that describes the relationship between the two parameters. The coefficients of this linear regression are a = 16.874 and b = –9.6488. The determination coefficient of this linear regression line is R^2 = 0.4685. The Pearson's correlation coefficient is r = –0.684 for α = 0.05. This means that a substantial negative connection exists between BLL and testosterone secretion in male, so the secretion of testosterone falls while the lead concentration in blood rises (from dose to dose).

Table 4 and Figure 4 illustrate the values and the connection that exist between lead accumulation in blood and LH secretion in blood in male *Guinea pig*.

Table 4

Concentration of lead in blood and LH in serum in male Guinea pig

Dose	Concentration	
	Pb (ppm)	LH (mIU/ml)
Control	0.000	0.16
TDI	0.147	0.22
Int. dose	1.173	0.20
LD_{50}–5%	1.882	0.20

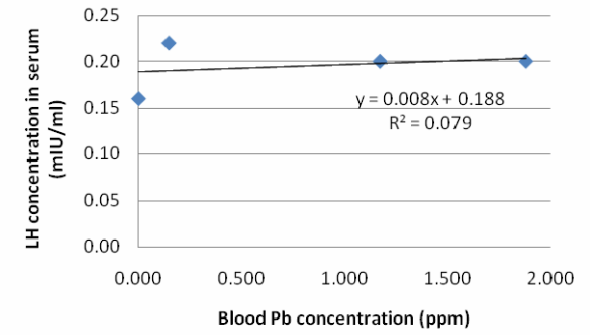

Fig. 4. Statistical connection between BLL and LH levels in serum in male *Guinea pig*

As it can be seen in Figure 4, the line of linear regression that determines the function of the relationship between BLL and LH secretion in males appears almost horizontal (a = 0.1886 and b = 0.008) and has a very small determination coefficient R^2 = 0.0795. The Pearson's coefficient results very small too, r = 0.282 for α = 0.05. This means that the connection between the blood level and LH secretion is very weak and the rise of BLL almost does not affect the secretion of LH by the pituitary gland in males.

The situation is different for the FSH secretion profile in males treated with lead (Table 5 and Fig. 5).

Table 5

Concentration of lead in blood and FSH in serum in male Guinea pig

Dose	Concentration	
	Pb (ppm)	FSH (mIU/ml)
Control	0.000	0.000
TDI	0.147	0.000
Int. dose	1.173	0.000
LD_{50}–5%	1.882	0.100

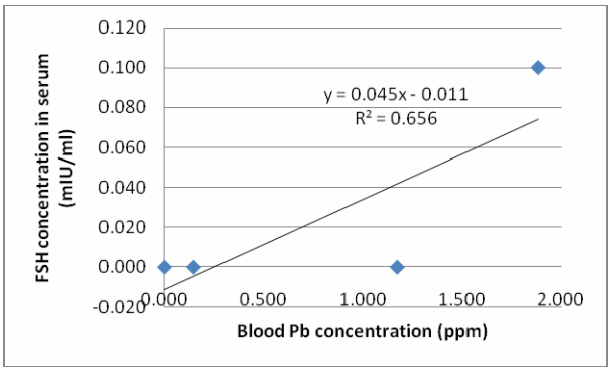

Fig. 5. Statistical connection between BLL and FSH levels in serum in male *Guinea pig*

The line of linear regression for the connection between lead accumulation in blood and FSH secretion is described by the function y = 0.0455x–0.0114 with determination coefficient R^2 = 0.6566. The Pearson's coefficient r = 0.81 for α = 0.05 confidence level means that the connection is statistically significant, a strong positive connection. The rise of BLL in males induces the rise of FSH secretion from the pituitary.

As an overall picture, from these results of lead effects in gonadotropic hormones and sexual steroid hormones secretion in males, it can be said that estradiol and progesterone secretion under the affect of lead are almost the same with significant increases. Instead the testosterone secretion decreases as a consequence of BLL increase and the absolute value of correlation coefficient is similar to that of the estradiol and progesterone (Rodami-

lans et al., 1988; Cullen et al., 1984; Braunstein et al., 1978; Alexander et al., 1998a). Lead in males results of no significant effect in LH secretion, but exerts a strong positive effect in FSH secretion by the anterior pituitary (Ng et al., 1991; Cullen et al. 1984).

Table 6

Summary of correlation coefficients Pearson's for the profile of each hormone secretion in correlation with lead accumulation in blood, according to doses applied during 60 days of treatment

Hormone Metal	Estradiol	Progesterone	Testosterone	LH	FSH	Hormone Dose
	0.3224	–0.7823	0.7794	0.0578	——	TDI
Pb ♂	0.8023	0.3982	–0.8268	–0.3058	——	Int. dose
	0.6727	0.3249	–0.8697	0.1801	0.9315	LD_{50}–5%

In better parts of working groups, different doses of the metal (which means different accumulation profile of metal accumulation in blood) exert different effects in hormone secretion. In TDI group the effect seems to be weaker and in different direction, when comparing with the two higher doses of lead but there are cases when the correlation is statistically significant, for example; progesterone and testosterone secretion.

Low dozes increase testosterone concentration in blood (r = 0.78, α = 0.05) while high dozes decrease it (intermediate dose r = –0.83, α = 0.05 and LD_{50}–5%, r = –0.87, α = 0.05). The secretion of FSH increases quickly with the application of the highest dose (r = 0.93, α = 0.05). The large increase of FSH secretion while testosterone decreases is an evidence of weakening of Leydig cell function (this was confirmed by the histological examination of the testicles).

All these data explain the inconsistent results of different authors, since the doses applied or BLL in casual toxication have been of different levels.

4. CONCLUSIONS

- Lead affects greatly the secretion of gonodotropins and sexual steroid hormones, increasing or decreasing their secretion.
- Different doses of lead have different effects in the secretion of the same hormone, especially when TDI dose is compared with the intermediate and LD_{50}–5% dose.
- TDI dose of lead has a weak effect in hormone secretion, but in some cases significant correlations emerge between BLL and hormone secretion (for example progesterone and testosterone in males treated with lead).
- High doses have great effects in hormone secretion and this is obvious when seeing the large values of correlation coefficients (positive or negative).
- The correlation between BLL and hormone secretion levels at the end of the treatment period, results positive for estradiol (r = 0.697), progesterone (r = 0.649), LH (r = 0.282) and FSH (r = 0.81), and negative for testosterone (r = –0.684).

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