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Down-regulation of CatSper 1 and CatSper 2 genes by lead and mercury

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High light

Administration with either lead acetate or mercury chloride caused degenerative damage in seminiferous tubules and reduction in sperm quality and expression of CatSper 1, 2 genes in m

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ABSTRACT

In the study of the expression of CatSper genes, consideration of the effects of environmental metal toxicity is very important. Therefore, in this study, the effects of lead acetate and mercury chloride exposure on expression of CatSper genes, sperm parameters, histology of testis and prooxidant antioxidant balance (PAB) values of serum were investigated.

A total of 28 mice was divided into four groups. The control group did not receive injections. The sham group received normal saline intraperitoneally. Lead and mercury groups were injected 60 and 1.25 mg/kg/daily lead acetate and mercury chloride respectively intraperitoneally for 2 weeks. After 35 days, the sperm analysis and histology of left testis were performed. In addition, serum was obtained to measure the PAB values. The right testis was used for molecular analysis of real-time PCR.

Administration with either lead acetate or mercury caused significant damage to the seminiferous tubules as well as a reduction in sperm parameters compared to the control group. The relative expression of CatSper 1 and CatSper 2 in the lead group was lower than that of the control group (-0.01 ± 0.24, -.007 ± 0.52 vs. 1 ± 0.50, P =0.34). The relative expression of CatSper 1 and CatSper 2 was significantly lower in the mercury group compared to the control ones (-0.24± 2.28, -4.49± 4.86

vs. 1 ± 0.50, P =0.21). PAB values significantly increased in lead or mercury exposed- mice compared to the control ones (0.93 ± 0.17, 1.54 ± 0.17 vs. 0.51 ± 0.11; P≤ 0.000).
The results of this study showed that administration with either lead acetate or mercury chloride caused degenerative damage in seminiferous tubules and reduction in sperm quality and expression of CatSper 1, 2 genes in mice. Therefore, it is possible in infertile men who have had exposure to lead acetate or mercury chloride. Owing to structural similarities, these metals are substitutes for calcium ions and have effects on calcium channels. These cause immobility in sperm by blocking CatSper-specific calcium channels. However, more studies are required to elucidate the mechanism underlying the impact of different doses of heavy metals on CatSper genes expression.
Key words: lead, mercury, CatSper gene expression, mouse

1. Introduction

Around 8% of couples suffer from infertility in our country (Safarinejad et al., 2008) .Almost half of these infertility cases are related to male infertility due to various reasons such as genetic mutations, infectious diseases, varicocele or environmental factors (Gil-Guzman et al., 2001) .Environmental factors like exposure to lead, mercury, formaldehyde, cadmium and nickel (Hsu et al., 2002; Mohammadi et al., 2014 ; Sadeghi et al., 2013; Mohammadi et al., 2015; Mohammadi et al., 2014) . Lead is one of the most important environmental pollutants that lead to adverse effects on the body's tissues (Hsu et al., 2002; Mohammadi et al., 2014 ; Sadeghi et al., 2013; Mohammadi et al., 2015; Mohammadi et al., 2014) . Pollution with lead is not only via fumes from gasoline cars but also, in the battery industry, solder, paint and painting supplies, protective screens, computers, televisions and optical fibre. People are affected by lead by breathing contaminated air and consuming contaminated food and water (Patrick et al., 2006; Mohammadi et al., 2016). Mercury is a heavy metal that can enter the body through food, water and air. Different pharmaceutical products, creams, soaps, cosmetics, amalgams in dentistry, children's toys and agricultural fungicides all contain mercury. Also, workers wearing felt hats have an occupational exposure to this compound. It has been reported that exposure to heavy metals causes harmful effects on the reproductive system (Shannon et al., 2007). Exposure to lead or mercury causes testis weight loss, decrease in seminiferous diameters, and a reduction in levels of antioxidant enzymes in testis tissue (Ayinde et al., 2012; Graça et al., 2004; Shan et al., 2009; Daniela et al., 2011; El-Desoky et al., 2013; Boujbihaa et al., 2009; Kalender et al., 2013). In addition, reductions in the number, motility and percentage of live sperm were observed (Ayinde et al., 2012; Graça et al., 2004; Shan et al., 2009; Daniela et al., 2011; El-Desoky et al., 2013; Pandya et al., 2012). Human studies show that

workers exposed to lead and infertile patients have a high serum level of heavy metals as well as a reduction in sperm parameters (Bonde et al., 2002; Benoff et al., 2009). In addition, it has been observed that exposure to lead or mercury caused changes in gene expression of key genes involved in spermatogenesis and antioxidant enzymes (Rodríguez-Estival et al., 2013; Wanga et al., 2013; Zhang et al., 2016; Xiaozhong et al., 2010; Martinez et al., 2016) . On the other hand, the study of calcium channels in sperms has led to the discovery of four genes, named CatSper 1-4, and two auxiliary proteins, CaSper ß and CatSper ɣ (Darszon et al., 2006) , which have a code for a unique sperm specific cation channel expressed exclusively in the spermatozoa (Qi et al., 2007). CatSper genes are required for sperm mobility and male fertility (Qi et al., 2007; Mohammadi et al., 2009). Owing to its crucial role in fertility and its restricted localization, the family of CatSper genes is predicted to be an excellent target for contraception and potential target for male infertility screening (Qi et al., 2007). With our survey, the effects of environmental factors on CatSper genes expression were not evaluated. Hence, we tried to answer the following questions: First, does administration with either lead acetate or mercury chloride change expression of CatSper genes in mouse testis? Second, does administration with either lead acetate or mercury chloride change sperm motility in adult male mice? Third, does administration with either lead acetate or mercury chloride change prooxidant antioxidant balance values in mouse serums? To the best of our knowledge, this is the first study to investigate the effects of lead acetate and mercury chloride treatment on CatSper genes expression and, therefore, we designed this study to evaluate the effects of lead acetate and mercury chloride administration on CatSper genes expression, sperm motility and PAB values in adult male mice.

2. Materials and Methods

Study design

After approval of this study by the Ethical Committee of Gonabad University of Medical Sciences, 28 male NMRI mice, 2 months of age, were used in this study. Animals were stored under standard environmental conditions (temperature 23–25°C, 55–50% humidity and 12-hour light-dark cycle). Mice were randomly divided into four groups of seven animals each (n = 7): (a) control, (b) sham group, (c) lead group, and (d) mercury group. Control groups received no supplementation. The sham

group received normal saline intraperitoneally. The lead group received 60 mg/kg/day lead acetate intraperitoneally for 2 weeks. The mercury group received mercury chloride intraperitoneally at 1.25 mg/kg daily for 2 weeks. After 35 days, animals were rapidly sacrificed by cervical dislocation, and the testis and cauda epididymis were immediately removed. Testis was stored at –80ºC until being used for RNA extraction and sperm suspension acquired from the epididymis was used for sperm analysis.

Chemicals

Lead acetate and mercury chloride ($Hgcl_2$) were obtained from Sigma-Aldrich Co (St. Louis, MO, USA).

Sperm analysis: Tail of epididymis sperm was separated and incubated at phosphate buffer saline at a temperature of 37 ° C. Then, sperm count, motility and normal morphology rate were evaluated, using a Neubauer haemocytometer (Rezazadeh Valojerdi et al., 2001).

Histological examination

After the fixation with formalin 10%, respectively dehydration with alcohol, transparency with xylene, infiltration with paraffin, moulding and then cutting were performed. Then, the slide was stained with H&E staining and a drop of entellan glue was poured on slides and covered with a cover glass.

Real-time PCR

After extraction of total RNA from tissue using Cinnagen protocol, reverse transcriptase reaction was performed by a Fermentas kit (Mohammadi et al., 2009; Mohammadi et al., 2013). After the reverse transcription reaction, in order to reproduce a given region of DNA, PCR reactions were performed on the product. The relative expression of CatSper1 and 2 CatSper genes were measured quantitatively using SYBR Green and β-actin gene. Real-time PCR reaction was carried out using Stratagene Max3000p instrument (USA). Data was analysed based on Pfaffi and colleagues' formula, as follows (Mohammadi et al., 2013; Pfaffl et al., 2001).

$$\text{Ratio} = \frac{(E_{\text{target}})^{\Delta CT\ \text{target}\ (control - sample)}}{(E_{\text{ref}})^{\Delta CT\ \text{ref}\ (control - sample)}}$$

Prooxidant Antioxidant Balance (PAB) assay

Blood samples were obtained from the mice hearts. After the blood clot, plasma was isolated in the 5000 rpm for 5 minutes. Sixteen mg of TMB powder was dissolved in DMSO solution and was added to the sodium acetate buffer (Solution 1). This solution was incubated with chloramine T for 2 hours. 25 units of peroxidase enzyme solution were added to Solution 1, 10 ml sodium acetate buffer, 200 ml of TMB/DMSO and 10 ml TMB. The final solution with 10 mL of sample and standard poured in ELISA plate reader and, after 10 minutes, 100mM HCL was added to the wells and the absorbance read at 450 nm wavelength (Alamdari et al., 2008).

Statistical analysis

SPSS software was used for data analysis. $P < 0.05$ was considered significant. Data was analysed using ANOVA, followed by Tukey test. P-values less than 0.05 were considered significant.

3. Results

Histological examination

Histological examination of the lead treated cases revealed mild edema, congestion, leydig cell hyperplasia and degrees of disintegration of germ cells from basement membrane (fig1. A). Mercury treated rats showed edema, apoptosis, disintegration of germ cells from their basement membrane, as well as decrease in the thickness of germ cell epithelium in some seminiferous tubules (fig1. B).

Effects of administration with lead or mercury on sperm quality in the NMRI male mice

Data was summarized in Table 1. There was a remarkable difference in sperm parameters between the lead and control groups (P≤0.001). Administration with 1.25 mg/kg mercury chloride caused a significant reduction in sperm count and motility (P ≤ 0.001; Table 1). The percentage of normal sperm morphology was affected by the lead or mercury group compared to the control group. Neck and tail abnormality (30.25%) was the most common abnormality in lead group and in the mercury group (29.75%).

Effects of lead acetate and mercury chloride on prooxidant antioxidant balance in the adult male mice

There was a significant increase between the prooxidant antioxidant balance (PAB) values in lead group compared to the control group (0.93 ± 0.17 vs. 0.51 ± 0.11; P ≤ 0.000). Also, a significant increase in PAB values was observed in mice treated with lead compared to the control group (1.54 ± 0.17 vs. 0.51 ± 0.1; P ≤ 0.000).

Effects of lead acetate administration on CatSper 1 and CatSper 2 genes expression in adult male mice

The relative intensity of CatSper genes expression is presented in Figure 2. The relative expression of CatSper 1 in the lead group was lower than that of the control group (-0.01 ± 0.24 vs. 1 ± 0.50, P = 0.34). The relative expression of CatSper 2 in the lead-treated mice was lower than that in the control mice (-.007 ± 0.52 vs. 1 ± 0.50, P = 0.78).

Effects of mercury chloride on CatSper 1 and CatSper 2 genes expression in adult male mice

As shown in Figure 3, the relative expression of CatSper 1 was significantly lower in the mercury group compared to the control ones (-0.24± 2.28 vs. 1 ± 0.50, P = 0.21). The expression of CatSper2 dramatically decreased in the mercury group compared to the control group (-4.49 ± 4.86 vs. 1 ± 0.50, P = 0.02).

4. Discussion

This study shows that administration of 60 mg/kg lead acetate or 1.25 mg/kg mercury chloride decrease prooxidant antioxidant balance values in the adult male mice. Damage to seminiferous tubules as well as the impairment of sperm parameters was observed after treatment with either lead acetate or mercury chloride. Similar to our results, Shan et al. reported that intraperitoneal injection of 1 mg/kg lead for 3 weeks decreased sperm count and motility compared to the control group (Shan et al., 2009). Anjum and colleagues reported weight loss, a reduction in sperm count and motility after lead acetate administration for 45 days (Anjum et al., 2011) . Pandya administered a dose of 0.02 mg/kg lead and cadmium intraperitoneally to the rats for 15 days. Increase in ROS, a decrease in antioxidant enzyme activity of testis tissue, and a significant reduction in the number, motility and viability of sperm were found (Pandya et al., 2012).

Ayinde in 2012 reported that the administration of 60 mg/kg lead acetate for 6 weeks causes degenerative changes in seminiferous tubules as well as a reduction in the thickness of germinal epithelium. Semen analysis showed a decrease in sperm count and morphology in lead exposure rats (Ayinde et al., 2012). In another study, 0, 50 and 100 ppm $Hgcl_2$added to weredrinking water of rats for 90 days. Detachment and degenerative changes in seminiferous tubules and reduction in the number and motility of sperm were observed on Day 7 after administration (Boujbihaa et al., 2009). Administration of 1mg/kg mercury chloride for 4 weeks cause increases as well as reductions in the levels of superoxide dismutase and glutathione peroxidase in rats. In addition, necrosis, tissue oedema, and disintegration of the basement membrane and spermatocytes were observed, compared to the control group (Kalender et al., 2013). In another study, 5 mg/kg $Hgcl_2$ was injected subcutaneously for 60 days. Levels of lipid peroxidation increased while glutathione peroxidase and catalase enzymes fell. Reduction in the number and motility were observed after administration of $Hgcl_2$. Apart

from interstitial haemorrhage, decrease in the tubular lumen and degeneration of spermatogonia were observed (El-Desoky et al., 2013).

In the present study, down-regulation of CatSper1 and CatSper 2 genes was found in mice exposed to lead and mercury. Besides, these heavy metals had a greater effect on the expression of CatSper 2 gene in the adult mice. Few studies have been performed on the effects of lead and mercury on the gene expression of the reproductive system. Benoff and colleagues, for instance, reported that calcium and potassium channels in sperm are very sensitive to toxic ions such as lead and cadmium and these cations—because of their structural similarity to calcium—cause devastating effects on the sperm. They concluded that this perhaps would be a reason for the high levels of cadmium and lead in semen of varicoceles and in infertile patients (Benoff et al., 2009). Wang and colleagues also investigated the effects of lead acetate at doses of 0.5, 1 and 1.5 g/l drinking water in male rat for 60 days. They found that this administration caused a remarkable reduction in the expression of Ddx3y, a key gene in the process of spermatogenesis (Wanga et al., 2013. Zhang and colleagues reported that the administration of 15 and 30 µg/L mercury chloride for 30 days caused a reduction the expression of fshr, lhr, ar, cyp17 and cyp11b in the testes, as well as an increase in mRNA expression of superoxide dismutase and catalase (Zhang et al., 2016) . Martinz and colleagues reported that Mercury—even at low doses—accumulated in reproductive organs such as the prostate, epididymis and testis, and increased expression of glutathione peroxidase enzyme (Darszon et al., 2006) . It seems that the mechanism of heavy metals is like lead or mercury as, because of their structural similarity to calcium ions, are replaced by calcium and these cause blocking of calcium channels—especially CatSper—as specific calcium channels. Another mechanism is increase in reactive oxygen species with the entry of toxic metals such as mercury and lead. Our results also showed an elevation in PAB values in experimental groups compared to the control group.

A limitation of this study was the investigation effects of only a dose of lead acetate and mercury chloride. It was better that it evaluates different doses of heavy metals on CatSper genes expression, but this was not possible due to limited financial support. Besides, it is better if mice are exposed to lead acetate or mercury chloride instead of intra-peritoperitoneal injection as a model of testicular injury.

Conclusion: The results of this study showed that administration with either lead acetate or mercury chloride caused degenerative damage in seminiferous tubules, reduction in sperm quality, and expression of CatSper 1, 2 genes in mice. Therefore, it is possible in infertile men who have had exposure to lead acetate or mercury chloride: due to structural similarities, these metals substitute with calcium ions and affect calcium channels and cause immobility in sperm via blocking of CatSpers as specific calcium channels. However, more studies are required to elucidate the mechanism underlying the impact of different doses of heavy metals on expression of CatSper genes.

Conflict of interest

None

Acknowledgment

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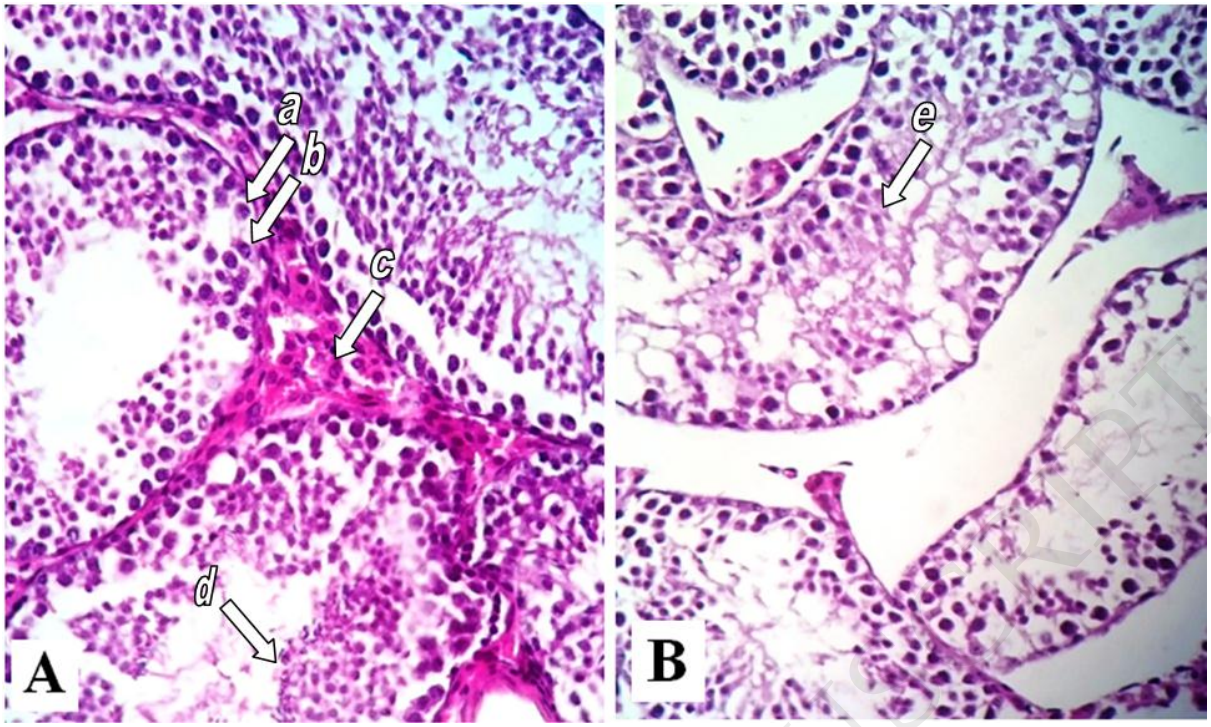

Figure1. Testis biopsies from the lead and mercury treated rats. A. In this case of lead treated rat there is mild congestion, leydig cell hyperplasia as well as disintegration of germ cells from basal membrane, a: spermatogonium, b: sertoli cell, c: leydig cells, d: spermatozoa (H&E, X400). B. Edema, disintegration of germ cells from basement membrane, apoptosis and decrease in the thickness of germinal epithelium is evident in this mercury treated rat , e: necrotic and apoptotic cells (H&E, X400).

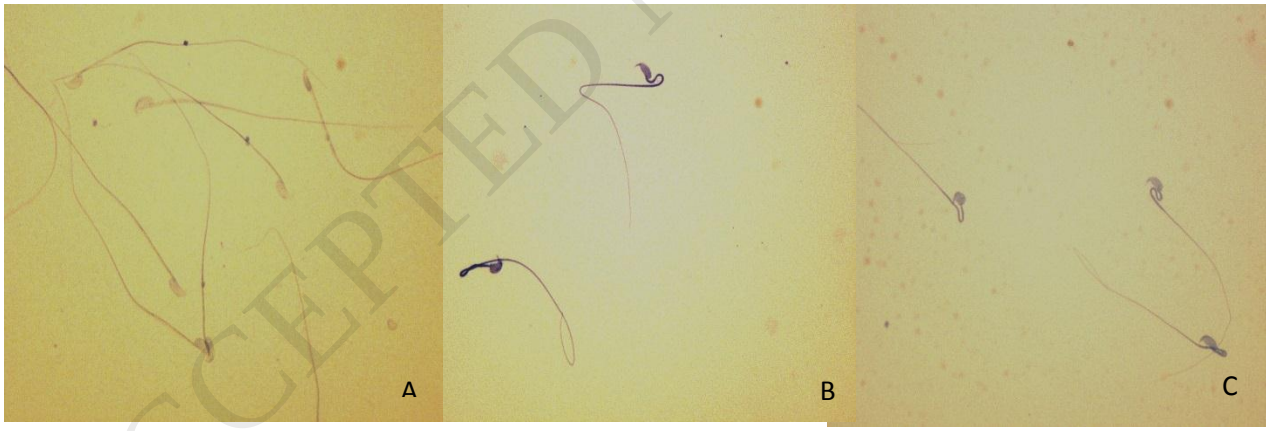

Figure 2. Images of sperm in control group (A), lead group (B) and mercury group (C).

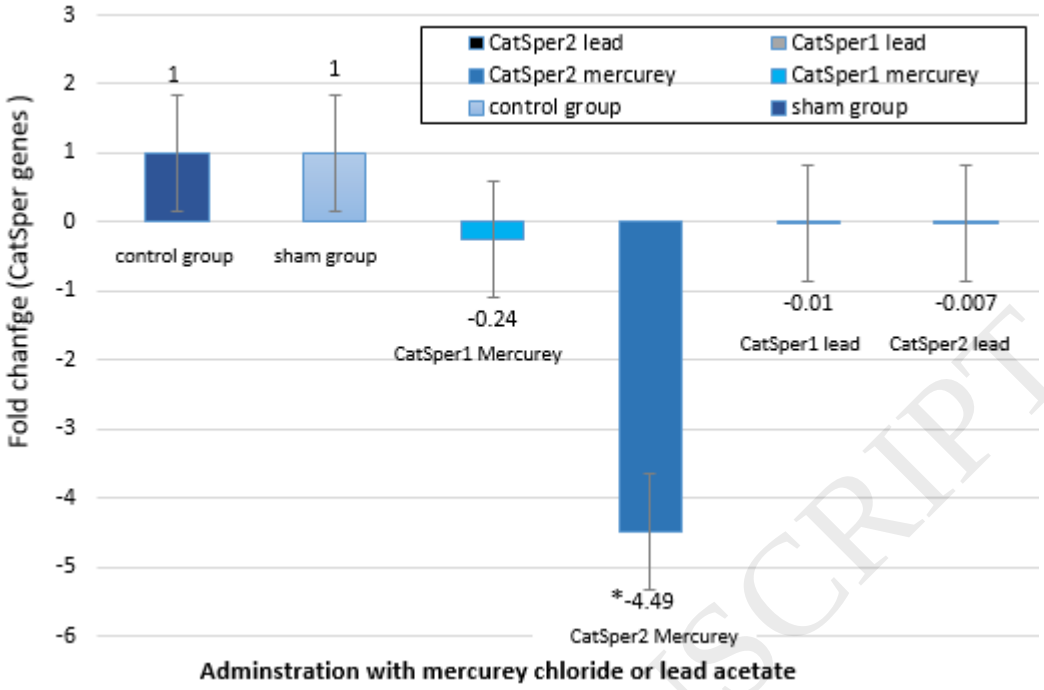

Figure 3. Comparison the relative expression of CatSper 1, 2 /ß-actin gene in control, mercury and lead groups: (n = 7 mice in each group).

Statistical analysis showed a significant difference between CatSper 2 gene expression in mercury group compared to the control group; * P = 0.02. The negative values mean a decrease in gene expression.

Table 1- Effect of lead acetate and mercury chloride on sperm quality in NMRI male mice

Sperm parameters	Control group	Sham group	Lead group	Mercury group
Sperm Count (million/mL)	4.50± 0.40	4.56± 0.25	3.50± 0.38*	3.40± 0.35*
Sperm motility (%)	68.00± 9.11	71.00± 4.20	48.66± 3.15*	43.5± 4.59*
Normal morphology rate (%)	88± 3.62	86± 4.10	69.75± 3.51*	70.25± 2.18*

Data are expressed as mean ± standard deviations of seven mice in each group. ANOVA test used for data analysis * $P \leq 0.000$ compared to the control group