



Effects of sub-chronic aluminum chloride on spermatogenesis and testicular enzymatic activity in male rats

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ABSTRACT

Aims: The aim of this study was to determine the effects of sub-chronic aluminum chloride (AlCl_3) on spermatogenesis and testicular enzymatic activity in male rats.

Main methods: Forty Wistar male rats were randomly divided into four groups: control group (CG, 0), low-dose group (LG, 64.18 mg/kg BW AlCl_3), mid-dose group (MG, 128.36 mg/kg BW AlCl_3) and high-dose group (HG, 256.72 mg/kg BW AlCl_3). The rats were orally administered with AlCl_3 for 120 days. At the end of the experiment, the contents of Al, Fe, Cu and Zn, the enzyme activities of testicular acid phosphatase (ACP), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), lactate dehydrogenase isoenzyme (LDH-x), the sperm count and the sperm malformation rate were examined.

Key findings: The results showed that the Al and Cu contents, sperm count and the enzyme activities of testicular ACP, SDH, LDH and LDH-x decreased, while the Zn and Fe contents and sperm malformation rate increased in AlCl_3 -treated rats.

Significance: It suggests that sub-chronic AlCl_3 disorders the balance of trace element and decreases the spermatogenesis and the activities of testicular enzymes, indicating that AlCl_3 has adverse effect on the testicular function in male rats.

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Introduction

The residual of available aluminum (Al) in biological ecosystems has been substantially increasing during recent years (Rengel, 2004; Fewtrell et al., 2009). Al exposure exerts adverse effects on the male reproductive system (Moselhy et al., 2012). Thus, Al was a potential risk for the reproductive function of men. After long time exposure, Al was accumulated in the testis (Guo et al., 2009) and induced marked lesions in seminiferous tubules by histopathologic examination (Yousef and Salama, 2009). Though some studies focused on the male reproductive toxicity induced by Al, the effects of Al on the spermatogenesis and the testicular enzymatic activity are still necessary to be clarified.

Coefficients of testis and epididymis are the ratio of testicular or epididymal weights and the body weight (BW). The BW and coefficients of testis and epididymis indicate the change of male reproductive function (Christian et al., 2001; Fang et al., 2012). Al decreased the BW and the

weights of testis or epididymis in mice and rabbits (Llobet et al., 1995; Johri et al., 2011), but effects of AlCl_3 on the coefficients of testis and epididymis are not clear.

Sperm count and sperm malformation rate reflect the reproductive function in male animals and humans (Cikutovic et al., 1993; Xia et al., 2009). Sperm count was the content of sperm in a given volume of seminal fluid. Sperm malformation rate was a ratio of the abnormal sperms to the total sperms. The effects of Al on the sperm count and sperm malformation rate are elusive. AlCl_3 decreased sperm concentration and motility while dead and abnormal sperm increased (Yousef and Salama, 2009). However, the sperm in cauda epididymidis and the percentage of morphologically abnormal sperm did not change in the Al ammonium sulfate-treated rats (0, 50 and 500 ppm) (Hirata-Koizumi et al., 2011).

Acid phosphatase (ACP), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH) and lactate dehydrogenase isoenzyme (LDH-x) can be used as markers for predicting testis function (Zhang et al., 2012; Pandey and Singh, 2002; Sinha et al., 1997). ACP catalyzes the hydrolysis of various phosphate esters in pH-optimum with the acid zone (Miteva et al., 2010). SDH transfers the fructose into sorbic alcohol and glucose which provides energy to sperms (Zhang and Lin, 2009). LDH plays an important role in testis energy production and biotransformation (Zhang and Lin, 2009). The inhibition of LDH-x activity induces

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denaturalization of spermatogenic cells (Sinha et al., 1997). The declined ACP activity was observed when rabbit sperm was incubated with different concentrations of AlCl_3 (0, 1, 5, 10, 15 and 20 mM) with or without vitamin C and vitamin E for 2 and 4 h (Yousef et al., 2007). Few data showed effects of Al on the activities of SDH, LDH and LDH-x in testis.

Therefore, this experiment examines effects of AlCl_3 on coefficients of testis and epididymis, trace elements (Fe, Cu and Zn) in testis, spermatogenesis (the sperm count and sperm malformation rate) and the enzyme activities (ACP, SDH, LDH and LDH-x) of testicular in the rats.

Material and methods

Rats

Forty healthy male Wistar rats (5 weeks old) weighed 110–120 g were randomly allocated into four groups ($n = 10$): control group (CG, 0), low-dose group (LG, 64.18 mg/kg BW AlCl_3), mid-dose group (MG, 128.36 mg/kg BW AlCl_3) and high-dose group (HG, 256.72 mg/kg BW AlCl_3). Rats were orally exposed to different doses of AlCl_3 in drinking water for 120 days. The dosages of AlCl_3 were determined according to Sun et al. (2011). All the rats were acclimatized for one week before the experiment.

Rats were housed in the Biomedical Research Center, Northeast Agricultural University. The housing conditions were maintained at temperature of $24 \pm 1^\circ\text{C}$, relative humidity at $55 \pm 5\%$, ventilation frequency at 18 times/h and a 12-h light/dark cycle. The rats were kept in plastic cages (five rats per cage) with soft chip bedding. The size of all the cages was $470 \times 300 \times 150$ mm, large enough for the growth of five rats. Throughout the experiment, chips were replaced every 3 days and rats were given drinking water and food ad libitum. The health status of rats was monitored daily and the BW of the rats was recorded every 10 days.

Sample collection

The experimental protocol was approved by the Ethics Committee on the Use and Care of Animals, Northeast Agricultural University, China. After 120 days, the BW of the rats was measured and the rats were sacrificed with ether vapor. The testes and epididymis were collected and weighed from each rat. Then the coefficients of testes and epididymis were calculated. Coefficients of testes and epididymis = testicular and epididymis weights (g) $\times 100\%$ /BW (g). Some of the testes were used to examine the contents of Al, Fe, Cu and Zn. Cauda epididymidis was freshly used to obtain sperm suspension solution to examine epididymal sperm count and sperm malformation rate. The rat sperm suspension preparation was performed according to Holloway et al. (1990). The testes were stored at -80°C to examine the enzyme activities of the ACP, SDH, LDH and LDH-x.

Determination of sperm count

Sperm count was calculated by hemocytometer according to Amaral et al. (2006). 10 μL of the sperm suspension was treated with water bath at 56°C to kill the sperm. Then the sperm count was calculated by the hemocytometer according the formula as follows: sperm count = the total sperm count in five medium square grid $\times 50,000 \times$ the volume of dilution solution (mL)/the weight of hemi-cauda epididymis (g).

Determination of sperm malformation rate

The sperm malformation rate was examined according to Liu et al. (2011). The spermatozoa dilution was mixed with 1% eosin solution in the ratio of 10:1. The stained time was 15 min–20 min. The glass slide was smeared, dried and fixed with methyl aldehyde for 10 min. The sperm malformation rate was calculated under microscope after the

glass slide was dried. 200 sperms were recognized. The abnormal sperm includes hook, larger head, curly head, double head and tail or without head and tail. The sperm malformation rate = the abnormal sperm count $\times 100\%$ /200.

Determination of ACP, SDH, LDH and LDH-x activities

The testes were processed with saline to obtain 10 mL of 10% testes tissue homogenate. The tissue homogenate were centrifuged at $10,000 \times g$ for 30 min. The supernatant of the tissue homogenate was collected. A part of the supernatant was used to detect the activities of ACP, SDH and LDH using ^{125}I radioimmunoassay (RIA) kits (New Bay Biological Technology Co., Ltd., Tianjin, China). The other part of the supernatant was heated with water bath at 65°C to lose the activities of ACP, SDH and LDH. Then the supernatant was used to detect the activity of LDH-x according to the detection method of LDH. The experiment procedure was followed by kit introduction.

The detection of Al, Fe, Cu and Zn in the testis

The concentration of Al in testis was determined in graphite furnace atomic absorption spectrophotometry, and concentrations of Fe, Cu and Zn were determined in flame atomic absorption spectrophotometry according to Zhu et al. (2012). Each testis was quantified 0.3 g and dried in the dryer (80°C). Then the samples were put into a triangle flask after 12 h drying, added 25 mL nitric acid and perchloric acid (volume ratio is 4:1), mixed and placed all night. The mixture was heated at a temperature range of 80 to 130°C about 5–6 min in an electric stove. When the mixture became transparent and the color was yellowish-brown, the digestion was over. After a period of cooling, the samples were transferred into a volumetric flask (25 mL), and constant volume with nitric acid (concentration is 0.5%). The Al standard solution was mixed with 1 mL Al standard stock solution (500 $\mu\text{g}/\text{mL}$) and 99 mL deionized water. The flame type was air-acetylene flame and determination line was 309.3 nm. The detection methods of Fe, Cu and Zn were the same as the method of Al. The determination lines of Fe, Cu and Zn were 248.3 nm, 324.7 nm and 213.9 nm, respectively.

Statistical analysis

Statistical analysis were done using SPSS 15.0 package programmer (SPSS Inc., Chicago, IL, USA). Data were shown as means and standard deviation (SD, bar on the top of each column). A P value of less than 0.05 was considered significant and a P value of less than 0.01 was considered markedly significant.

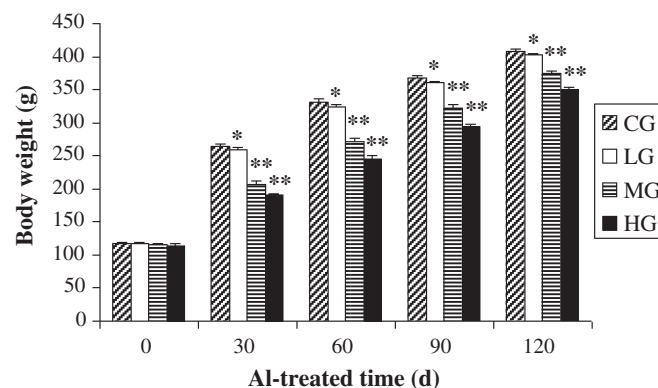


Fig. 1. Effects of Al exposure on the BW of rats. CG – control group, LG – low-dose group, MG – mid-dose group, and HG – high-dose group. * $P < 0.05$, ** $P < 0.01$ versus control group.

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