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Effects of sub-chronic aluminum chloride exposure on rat ovaries

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ABSTRACT

Aims: This experiment investigated the effects of sub-chronic aluminum chloride ($AlCl_3$) exposure on rat ovaries.
Main methods: Eighty female Wistar (5 weeks old) rats, weighed 110–120 g, were randomly divided into four treatment groups: control group (CG), low-dose group (LG, 64 mg/kg BW $AlCl_3$), mid-dose group (MG, 128 mg/kg BW $AlCl_3$) and high-dose group (HG, 256 mg/kg BW $AlCl_3$). The $AlCl_3$ was administered in drinking water for 120 days. The ovarian ultrastructure was observed. The activities of acid phosphatase (ACP), alkaline phosphatase (ALP), succinate dehydrogenase (SDH), Na^+–K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase, the contents of Fe, Cu and Zn, and the protein expression of follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) in the ovary were determined.
Key findings: The results showed that the structure of the ovary was disrupted, the activities of ALP, ACP, SDH, Na^+–K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase, the contents of Zn, Fe and the protein expression of FSHR and LHR were lowered, and the content of Cu was increased in $AlCl_3$-treated rats than those in control.
Significance: The results indicate that sub-chronic $AlCl_3$ exposure caused the damage of the ovarian structure, the disturbed metabolism of Fe, Zn and Cu and the decreased activities of Na^+–K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in the ovary, which could result in suppressed energy supply in the ovary. A combination of suppression of energy supply and reduction of expression of FSHR and LHR could inhibit ovulation and corpus luteum development, leading to infertility in female rats.

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Introduction

Biological effects of aluminum (Al) are linked to the development of dialysis dementia, osteomalacia, Alzheimer's disease, and Parkinson's disease. Our previous researches have shown that $AlCl_3$ caused toxic effects on the brain, bone, immune, and hematopoietic system (Gu et al., 2009; Li et al., 2011; Zhu et al., 2011; Zhang et al., 2011). Toxicity to the reproductive system of Al has also drawn great attention worldwide recently. Among these researches, most of them focus on the male reproductive system (Yousef et al., 2007; Sun et al., 2011; Ige and Akhigbe, 2012), while a few reports are about the female reproductive system. In female mice, Al accumulates in the ovary which could damage the ovarian structure (Chinoy and Patel, 2001). However, Al causes a transient disturbance to oestrous cycle regularity in female rats, but does not develop into reproductive toxicity (Agarwal et al., 1996). Sakr et al. (2010) reported an excess of congenital anomalies in women workers at an Al smelter compared with their previous employment periods without access to Al, and demonstrated that Al exposure has detrimental effects on the reproductive system.

In many developing countries, infertility is an important health issue (Esmaeilzadeh et al., 2013). A recent population-based-survey across many countries estimated the prevalence of infertility to be 9% on average, with a range of 3.5% to 16.7%, and female infertility is higher than that of the male (Boivin et al., 2007). Causes to infertility are multifactorial. Ovulatory disorders caused by disturbed hormone profiles and damaged ovary structure can lead to infertility (Kuohung et al., 2011). Trif et al. (2008) found that Al level was high in the ovarian and uterine tubes of adult female rats administrated orally with Al sulfate, and the tissue levels were correlated to the Al dosages. Shen et al. (1999) observed that Al nitrate at concentrations of 30 mg/L and 60 mg/L in the medium inhibited the viability and meiotic maturation of cultured mouse oocyte. Agarwal et al. (1996) found that the offspring of gestation rats treated with Al lactate disturbed oestrous cycle regularity. Wang et al. (2011) suggested that Al exposure disturbed the secretory function of the ovary, and reduced the levels of FSH and LH in the rat serum. These evidences demonstrate that Al is a potential risk to female infertility.

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We hypothesize that aluminum chloride ($AlCl_3$) can damage the ovary, leading to female infertility. We used a rat model to investigate the effects of oral administration of $AlCl_3$ on the ovary. In the experiment, the morphology of the ovary, the contents of trace elements (Fe, Cu and Zn), the activities of acid phosphatase (ACP), alkaline phosphatase (ALP), succinate dehydrogenase (SDH), Na^+–K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase, and the expression of follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) in the ovary were determined to depict the basic toxicological mechanisms of $AlCl_3$ on the functions of the ovary.

Materials and methods

Rats

Eighty healthy female Wistar rats (5 weeks old), weighed 110–120 g, were randomly allocated into four groups. All the rats were acclimatized for one week, and then $AlCl_3$ was orally administered in drinking water for 120 days. The concentrations of $AlCl_3$ in drinking water were 0 (control group, CG), 0.4 g/L (low-dose, LG), 0.8 g/L (mid-dose, MG) and 1.2 g/L (high-dose, HG), respectively. The water consumption of the individual rats averaged at 18 mL/d with a range from 16 to 19 mL/d, resulting in the doses of $AlCl_3$ at 64 (1/20 LD_{50}), 128 (1/10 LD_{50}), and 256 (1/5 LD_{50}) mg/kg BW $AlCl_3$ respectively for LG, MG, and HG groups. The doses of this experiment were determined according to the LD_{50} of $AlCl_3 \cdot 6H_2O$ (1283.60 mg/kg).

All rats had free access to fresh water and housed in cages at temperatures of 22–25 °C and relative humidity at 55 ± 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 the cages was $470 \times 300 \times 150\ mm^3$, which was adequate for accommodating five rats. Throughout the experiment, wood chips were renewed every third day and rats were given drinking water and food ad libitum. The health state of rats was monitored daily and the body weight was recorded monthly.

Sample collection

The use of the animals and the study protocol was approved by the Ethics Committee on the Use and Care of Animals, Northeast Agricultural University (Harbin, China). After they were administered with $AlCl_3$ for 120 days, the rats were anesthetized with ether. The ovary was collected, and 0.1 g of the ovary sample was weighed and homogenized in ice bath for detecting the enzyme activities. An ovary tissue sample about $1.0 \times 1.0 \times 2.0\ mm^3$ size was put into 10% formaldehyde fluid for the detection of FSHR and LHR. And $1.0 \times 1.0 \times 1.0\ mm^3$ ovary tissue was put into 2.5% glutaraldehyde solution for the observation of the ovarian morphology. The remaining ovary tissue was stored at −70 °C for the determination of Fe, Zn and Cu concentrations.

The detection of the ovarian morphology

The ovarian samples were fixed in 2.5% glutaraldehyde solution for 72 h. The samples were embedded in Spurr's resin by using Leica/LKB Embedding Capsules Easy Molds. Ultrathin sections with an average thickness of 70 nm were sectioned with a Reichert Ultracut equipped with a diamond knife, stained with uranyl acetate and lead citrate, and examined under a JEOL JEM-1230 electron microscope which was operated at 80 kV. A picture by model lesion was taken. This method proceeded according to Yamada et al. (2014).

Determination of Fe, Zn and Cu levels in the ovary

The levels of Zn, Fe and Cu in the ovary were determined in flame atomic absorption spectrophotometry by Zhu et al. (2011). The ovary was quantified to 0.1 g and dried at 80 °C for 12 h. The sample was then transferred into a triangle flask, added with 4 mL nitric acid and 1 mL perchloric acid, and kept overnight. The mixture was heated slowly till it became colorlessly transparent. After cooling at room temperature, the sample was transferred into a 5 mL volumetric flask, and the volume was made up to 5 mL with 0.5% nitric acid. The sample was protected from light during the process. The absorbency of the solution was read in a flame atomic absorption spectrophotometer (HP 3510 atomic absorption spectrophotometer). The Zn, Cu and Fe standard solutions were prepared in 0.5% nitric acid for the derivation of the calibration curves for each element. The wavelengths were respectively set for recording Zn, Fe and Cu at 213.9 nm, 248.3 nm and 324.7 nm.

Determination of enzyme activity in the ovary

The activities of ALP, ACP, SDH, Na^+–K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in the ovary were determined using ^{125}I radioimmunoassay kits (New Bay Biological Technology Co. Ltd., Tianjin, China), following the procedure of the kit introduction.

Determination of FSHR and LHR expressions in the ovary

FSHR and LHR expressions were determined by immunohistochemistry (Guo et al., 2005). The fixed ovary sample was embedded with paraffin, and then stained with FSHR/LHR immunohistochemistry kits (Beijing Biosynthesis Biotechnology Co. Ltd., Beijing, China/Wuhan Biosynthesis Biotechnology Co. Ltd., Wuhan, China). After deparaffinage, sections in 0.01 M citrate buffer solution (pH 6.0) were placed in microwave oven for 20 min to unmask the FSHR/LHR protein. The sections were incubated with endogenous peroxide blocking solution (0.3% H_2O_2) at 37 °C for 30 min. Thereafter, the sections were incubated for 30 min with serum taken from a rabbit which was not immunized by the target protein. This kind of protein would eliminate a false positive reaction. The samples were subsequently incubated with a rabbit anti-rat FSHR/LHR polyclonal antibody in 1:200 PBS (pH 7.4) at 4 °C overnight. Then the samples reacted with the biotin-conjugated second antibody and streptavidin-peroxidase solution for 1 h at room temperature, and a diaminobenzidine (DAB) solution was added to stain the FSHR/LHR protein. The average gray scale was measured using Motic image 3.2 micrograph analysis software (Motic, German), which was applied to quantitate the nuclear FSHR/LHR levels. The average gray scale was negatively correlated with the expression of FSHR/LHR protein. The sections were observed under a microscope (model: BA 400, Motic, German).

Statistical analysis

The results were expressed as least square mean ± standard deviation (SD) and analyzed by one-way analysis of variance using the statistical package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered a significant difference and $P < 0.01$ was a markedly significant difference.

Results

Ultrastructure of the ovary

The nuclear chromatin, the nuclear envelope, rough endoplasmic reticulum and the structure of mitochondria of the ovary are shown in Fig. 1 for CG, and Fig. 2 for HG. There were obvious damages on the nuclear and cytoplasm in HG. The margination and concentration of nuclear chromatin (Fig. 2B-1, C-1 and D-1) show the apoptosis of the ovary granulosa cells (Fig. 2B-a). The nuclear envelope structure is irregular (Fig. 2D-4). The mitochondria are swollen, the cristae are disintegrated and vacuolated (Fig. 2B-2, C-2 and E-2), the rough endoplasmic reticulum is dilated and loses ribosomes (Fig. 2D-3), and the Golgi body

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