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Effects of fluoride and aluminum on expressions of StAR and P450scc of related steroidogenesis in guinea pigs' testis

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HIGHLIGHTS

- F and Al changed histological structure of testes, reduced sperm quality.
- F and Al reduced StAR and P450scc expressions and level of serum testosterone.
- F toxicity is stronger than Al and Al weakened the toxicity of F.
- Al had antagonism effects on F.

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ABSTRACT

A lot of studies have shown that fluoride and aluminum have toxic effect on male reproductive system, but the mechanism of which and the interaction between fluoride and aluminum is still unknown. This study investigated the effects of fluoride (NaF) or/and aluminum ($AlCl_3$) on serum testosterone level, gene and protein expression levels of Steroidogenic Acute Regulatory Protein (*StAR*) and Cytochrome P450 cholesterol side chain cleavage enzyme (*P450scc*) in the testes of guinea pigs. Fifty-two guinea pigs were divided randomly into four groups (Control, HiF, HiAl and HiF + HiAl). Fluoride (150 mg NaF/L) or/and aluminum (300 mg $AlCl_3$/L) were orally administrated to male guinea pigs for 13 weeks. The results showed that F and Al reduced number and elevated abnormal ratio of sperm. Meanwhile, the concentrations of serum testosterone in all experimental groups were decreased. P450scc protein expression was significantly reduced in all treatment groups, and StAR expression was decreased remarkably in HiF group and HiF + HiAl group. The levels of StAR mRNA in three groups were reduced by 53.9%, 21.4% and 33.4%, respectively, while the expressions of P450scc mRNA were reduced by 67.8%, 17.0% and 47.8%. Therefore, we concluded that F induced the reduction in testosterone and sperm amount, and thus in lower fertility, which might occur as a consequence of depressed *StAR* and *P450scc* mRNA expression. There were no synergistic effects between F and Al, instead, Al weakened the toxicity of F to some extents. The results indicated that Al had antagonism effects on F.

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1. Introduction

Fluoride (F) usually exists widely in the environment as inorganic or organic compounds due to its great reactivity. A lot of studies have shown that excessive exposure to F may not only result in detrimental effects on skeleton and teeth (Everett, 2011), but also other soft tissues, such as liver, kidney, brain, and spinal cord (Perumal et al., 2013; Sun et al., 2011). Moreover, a lot of evidence indicated that F interfered with the reproductive system, including the reduced number of sperm, and the elevated abnormal ratio of sperm, the disturbed spermatogenesis, the remarkable reduction of testosterone (Lu et al., 2014; Pushpalatha et al., 2005;

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Zhang et al., 2006). However, the cellular mechanisms underlying the reproductive toxicity of F are unclear.

Drinking water is the primary source of F exposure in humans, in which fluoride coexists with several other xenobiotics, frequently metals (Barbier et al., 2010). Many studies have shown that minerals are closely related with the secretion of sex hormone, sexual function, and reproductive diseases of men and male mice (Colagar et al., 2009; Croxford et al., 2011; Sarosiek et al., 2009). Thus, the minerals play a significant role in the reproductive function.

Aluminum (Al) is the third most common element in the earth's crust. People can absorb/accumulate Al via the diet or drinking water. Study has reported that even a low dose of Al could inhibit the adverse effects of F (Lubkowska et al., 2006). However, Al could lead to Alzheimer's disease and excessive amount of aluminum resulted in the accumulation in target organs, which had a link with the damage of testes of both humans and animals (Yousef, 2004; Yousef et al., 2005). The combined effects of F and Al may be somewhat mixed and need to be clarified.

Reproductive system, which is involved in the continuation of the species, plays an important role in the whole life of organisms. Sex steroid hormones secreted mainly in the gonads are important for the gametogenesis, gonad development and maturation in the reproductive process (Kime, 1993). Testosterone, one of the most important steroid hormones produced in the testis, is vital for the initiation of spermatogenesis (Guan et al., 2012; Sun et al., 2010; Zhang et al., 2006). The production of steroid hormones is initiated by translocation of cholesterol from the outer to the inner mitochondrial membrane, an event that has recently been found to be mediated by the steroidogenic acute regulatory protein (StAR) (Hasegawa et al., 2000; Luo et al., 2005; Prasad et al., 2015; Shen et al., 2003), and the subsequent conversion of cholesterol to pregnenolone on the inner membrane of the mitochondria, the site of cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc), is considered to be the primary points of control in steroidogenesis (Augustine, 2008; Saez, 1994). Thus it can be inferred that StAR and P450scc play an important role in the process of testosterone biosynthesis. However, there are few studies on the toxic effects of F or Al, or the effects of co-exposure to F and Al on the expressions of StAR and P450scc.

The present study aimed to investigate a possible mechanism of F-induced testis toxicity and the role of Al in disturbing the toxic effect of F on testes of guinea pigs by measuring the testosterone in blood and the mRNA and protein expression levels of StAR and P450scc in their testes.

2. Materials and methods

2.1. Materials and chemicals

Serum testosterone radioimnunoassay kit was obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). StAR polyclonal antibody was obtained from Uscn life Science & Technology Company (Wuhan, China). P450scc polyclonal antibody and Immumohistochemistry Kit were purchased from Wuhan Boster Biological Technology, Ltd. (Wuhan, China). Trizol was purchased from Invitrogen (Carlsbad, CA, USA). SYBR® Premix Ex Taq™ II QRT-PCR kit was obtained from Takara Biotechnology Company (Dalian, China). All the real-time PCR primers were synthesized by Beijing AuGCT biotechnology Co., Ltd (Beijing, China). The other reagents used were analytical grade.

2.2. Animals and treatment

Fifty-two adult male guinea pigs with the body weight of 250–300 g were obtained from Experimental Animal Center of Xinglong in Beijing, China. All guinea pigs were maintained on a standard laboratory diet at 22–25 °C under a 12/12 h light/dark cycle with adequate ventilation and proper and hygienic conditions. The guinea pigs were divided randomly into four groups of 13 each: a control group, which was received distilled water, HiF group, HiAl group, and HiF + HiAl group which were given NaF 150 mg L^{-1}, $AlCl_3$ 300 mg L^{-1}, NaF 150 mg L^{-1} + $AlCl_3$ 300 mg L^{-1} in their drinking water, respectively. Body weights of guinea pigs were measured at 0, 2, 4, 6, 8, 10, 12, 13 weeks. After 13 weeks, all guinea pigs were injected with 20% urethane solution for fatal anesthesia. Blood samples were collected for testosterone measurement. The left testis was carefully removed and rinsed with distilled water to remove blood. Each testis was cut into two pieces, one piece was fixed in 10% neutral formalin for histopathological examination and immunohistochemical analysis, the other piece was snap frozen by liquid nitrogen and then stored at −80 °C for gene expression assay. The left epididymis was collected for determination of sperm density, sperm motility and rate of teratosperm. This work was approved by the Institutional Animal Care and Use Committee of Shanxi Agricultural University.

2.3. Evaluation of sperm quality

The sperm suspension of left epididymis was prepared in normal saline at 37 °C. Sperm motility, rate of teratosperm and sperm density were calculated by the method of Prasad et al. (1972) and expressed as percentage and density as $\times 10^6$/L, respectively.

2.4. Histopathological examination

After being immersed in 10% neutral formalin for 16 h, testes samples were again rinsed with distilled water, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. Finally, they were cut into 4-μm sections with a rotary microtome and stained with hematoxylin and eosin.

2.5. Measurement of testosterone in serum

Blood samples were centrifuged at 1400× g for 10 min at room temperature. The supernatants were aliquoted into Eppendorf tubes. The samples were detected using γ Radiation immunization register (USTC Zonkia Scientific Instruments Co. Ltd, Anhui, China) according to the instruction of serum testosterone radioimnunoassay kit.

2.6. Immunohistochemical analysis

4-μm sections of the testes were attached to slides treated with 3-Aminopropyl-Triethoxysilane (APES). After being deparaffinizated and rehydrated, sections were washed 2 times in PBS and then were exposed to 3% H_2O_2 for 10 min to block endogenous peroxidases, followed by rinsing in PBS for 5 min. Then sections were placed in a 0.01 M citrate buffer solution (pH 6.0) at 95 °C water bath for 10 min and cooled to room temperature. Subsequently, the sections were incubated with normal nonimmune serum for another 20 min. Afterward, incubation with primary antibody at 1:150 diluted with PBS (0.1 mol L^{-1}, pH 7.4) was performed at 4 °C over night. Next, a biotin-labeled goat anti-rabbit secondary antibody was introduced at a dilution of 1:1000, and incubation was conducted at 37 °C for 20 min. After washing in PBS, sections were visualized with 3.3′-diaminobenzidine (DAB) and counterstained with hematoxylin solution. Finally, the sections were dehydrated in ethyl alcohol and coverslipped. Optical density was calculated according to the color depth and area of dyestuff

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