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Effects of Aluminium Chloride Injection in Lateral Ventricle on Serum Gonadothropines, Testosterone and Spermatogenesis in Rats

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This study was designed to investigate the effects of aluminium in male Rat. The experiment was performed on four groups of male rats, with lateral ventricle cannulated. Test group received aluminium chloride in lateral ventricle. Two series received the same volume of (5.5 μL) of ACSF with pH = 3.4 and 7.2. Sham control did not receive any agent. At the end, serum FSH, LH and Testosterone in all groups were measured by RIA methods. Epididymis and vas deferense were dissected, cut, diluted and spermatozoa were counted. Data obtained were analysed by ANOVA and Tukey-test. The results were expressed as mean±SE and $p<0.05$ were considered significant. The results showed, FSH, LH, testosterone and spermatozoid count per gram of tissues in vas deferense and epididymis, were decreased significantly in the test group which received aluminium chloride compared with other groups ($p<0.05$). The results of this study indicate that toxic effect of aluminium can be exerted via central nervous system.

Key words: Aluminium, lateral ventricle, epididymis, FSH LH, testosterone

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INTRODUCTION

Aluminium is a potent voltage sensitive calcium channels blocker and enters into the body via skin, lung, gastrointestinal tract and medicine (Busselberg and Platte, 1994; Chen and Liu, 2005; Robert *et al*., 2006). This ion can be accumulated in the body tissues and impaired organelles (Domingo and Gomez, 1993; Jose and Roig, 2006; Greger and Radzawski, 1995). Long-term oral exposure to low doses of aluminium promotes alterations on erythrocyte parameters in rats (Niu, 2005; Farina and Rotta, 2005). Inter Peritoneal (IP) administration of aluminium salts in Rats, changed creatinin clearance and urine concentration of bivalent ion such as Mg^{++} and Ca^{++} (Liu and Norenberg, 1995). Aluminium poisoning can inhibit signal transduction in cell membrane (Haug and Shi, 1994; Jose and Rigo, 2006). The increases of serum aluminium alter the activity of the enzymes those having a metal element in their structures (Kaur and Gill, 2006). Aluminium poisoning could affect learning, memory and it is an important candidate for Alzheimer disease (Gupta and Anitha, 2005; Platt and Carpenter, 1995; Alessio *et al*., 1989). Aluminium mine workers, who have had high level of serum aluminium had shown decreased serum concentration of Thyroid-Stimulating Hormone (TSH) and prolactine (Agarwal and Ayyash, 1995; Rosenlof and Fyhrouist, 1990). Patients with renal failure under dialysis have high serum aluminium level and show impairment in reproduction (Yamamoto and Sofikitis, 1996). Application of trace elements (Pb^{++}, Zn^{++} and Al^{3+}) intracellularely have reduced Voltage-Activated Calcium Channels Currents (VACCCs) (Platt, 1994) Since GnRH synthesis, secretion and spermatogenesis dependent on calcium ion (Tse *et al*., 1993; Watanabe *et al*., 1994), this study was performed to show the effect of aluminium microinjection in rat's lateral ventricle (ICV) system in the rat.

MATERIALS AND METHODS

Male Sprague-Dawley, albino rats weight 235-347 g (Razi institute Tehran Iran), were housed in group cages under controlled conditions (temperature 22-28°C and 12/12 h light and dark cycle, light on at 6:00 am at least 10 days before the start of the experiments with free access for food and water. Experiments were performed in rats (n = 55) deprived of food for 24 h but given free access to water. Rats were weighted (first weight) and anaesthetized with IP injection of ketamin (150 mg kg^{-1}, Gedeon Richcer chemical works, Hungry). Each animal was implanted a cannula (21-1/2 stainless steel, outer diameter = 0.9 mm) in the lateral ventricle to deliver the solutions. With the help of a sterotaxic unit (Narishige Japan,) lateral ventricle was unilaterally implanted according to Paxinose Atlas (Incisor bar: -6 mm below the interaural; AP = +1.4 mm from bregma; DV = +3.4 mm from surface of the brain; ML = ±2 mm from midline) and fixed to the skull with two stainless steel screws and dental cement. Following a one week of recovery period animals were divided in four groups. Test group received 5.5 μL of Artificial Cerebra Spinal Fluid (ACSF) containing 4.125 pmole aluminium chloride (Merck Germany), pH = 3.4 using a Hamilton syringe twice a day for 20 days. Two groups of the animals received the same volume of ACSF with pH = 7.2 (effect of volume) or pH = 3.4 (effect of pH). The sham control animals receive any agent after cannulation (effect of cannulaton). At the end of the experiment, animals were anesthetized with sodium thiopental (Sepia Nesdonal) and vas deferens, epididymis and testis were removed and weighted. Vas deferens and epididymis were dissected, cut and diluted with normal saline. Spermatozoa were counted by hemocytometer and count was justified per gram of vas deferens and epididymis (Linde *et al*., 1994). Blood samples were collected through abdominal aorta, sera were separated and kept at -20°C until the time of the assays. FSH, LH and testosterone were measured by RIA methods. At the end and before sacrificing the animals, 5 μL thionin solution was injected into the guide cannula to locate the position of the cannula. The brains from the animals were removed and placed in fixator for 24 h. After post fixation, brains were sectioned and after assessing the position of the cannula the data only from those animals with cannula in right position were used in the analysis (n = 50). Results are expressed as mean±SE, ANOVA and Tukey-test were used to analyze the results and ($p<0.05$) were considered significant.

RESULTS AND DISCUSSION

Results showed that serum FSH, LH and testosterone concentrations were significantly decreased compared with those of the shame control group (Table 1). Vas deferens weight in the test group which received aluminium (119.5±2.4 mg) was significantly ($p<0.05$) decreased compared with the sham control group (130.8±2.4 mg (Table 2). Epididymis weight in test group was 490.3±8.45 mg, which was significantly decreased compared to those of the sham control group (569.6±13.75 mg (Table 2). Spermatozoid count per gram of vasa deferens in test group was 43.68±1.74×106 and this value was too significantly decreased compared to the sham control group (73.3±2.81×106) (Table 3). Testis weight in the test group was 1520±30 mg which is less than the control group (1720±30 mg) (Table 2).

Table 1: FSH, LH and testosterone in the study groups

	Groups				
Hormone concentration	Sham control	ACSF (pH = 7.2)	ACSF (pH = 3.4)	ACSF with 4.15 pmol aluinium	p-value
FSH (μU mL^{-1})	790±100*	710±190	870±160	510±100	0.03
LH (μU mL^{-1})	1920±180	1740±230	1640±120	1240±90	0.004
Testosterone (ng mL^{-1})	1.74±0.38	1.07±0.22	1±0.18	0.43±0.09	0.005

*Mean±SE, n = 13

Table 2: Vas deference, epididymis and testis (mg) weight in study groups

	Groups				
Hormone concentration	Sham control	ACSF (pH = 7.2)	ACSF (pH = 3.4)	ACSF with 4.15 pmol aluinium	p-value
Vase deference (mg)	120.8±2.15*	115.4±2.7	117.5±1.5	104.65±2.3	0.002
Epididymis (mg)	569.6±13.25	555±12.9	560±14.05	490±8.45	0.005
Testis (mg)	1720±20	1690±50	1710±50	1520±60	0.001

*Mean±SE, n = 13

Table 3: Spermatozoid count millions per gram of tissues in study groups

	Groups				
Hormone concentration	Sham control	ACSF (pH = 7.2)	ACSF (pH = 3.4)	ACSF with 4.15 pmol aluinium	p-value
Vase deference of	73.3±2.81	77.55±1.23	72.28±2.18	53.68±1.74	0.001
Epididymis	107.73±3.12	109.30±3.03	105.65±2.76	79.78±3.08	0.005

* Mean±SE, n = 13

Table 4: The first and final weight in study groups

	Groups				
Hormone concentration	Sham control	ACSF (pH = 7.2)	ACSF (pH = 3.4)	ACSF with 4.15 pmol aluinium	p-value
First weight (g)	283.92±9.24	296±7.67	291±5.25	292±6.38	
Final weight (g)	304±5.75	299.58±5.52	294.58±6.63	262.77±6.11	0.003

* Mean±SE, n = 13, NS: Not significant

Spermatozoid count per gram of epididymis tissues in test group was significantly decreased compared with those of the control group (Table 3). The weight of the group receiving aluminium at the end of the study period (292±6.36 g) was significantly ($p<0.05$) lower compared with the control group (304±5.8 g) (Table 4).

FSH, LH, testosterone concentrations, vas deference, epididymis, testis and body weight, Spermatozoid count per gram of vasa deferens and epididymis tissues were not significantly different among the sham control group and those who received only ACSF with pH = 7.2 or 3.4.

The result of this study showed that aluminium injection in rat's lateral ventricle, significantly reduces FSH, LH and testosterone concentrations as well as the spermatozoid count per gram of vas deferens, epididymis and the weight of these organs, testis and body weight. The results of this study may explain partly the toxic effect of the metal on the reproduction. The effect of aluminium injection centrally on the reproductive system has not been investigated. However it has been suggested that AlCl can exert a significant adverse effect on reproductive performance in the animal model (Yousef *et al*., 2005). This effect can be exerted centrally, peripherally or both. Aluminium is a Voltage Sensitive Calcium Channels (VSCC) blocker (1994) and can affect neurotransmitter release (Jones and Kochian, 1997). Aluminium injection in rat hippocampus has shown that, the rate of glutamate neurotransmitter release, significantly decreased compared to the control group (Platt *et al*., 1995). Since GnRH secretion and synthesis is strongly dependent on calcium and VSCC (Tse *et al*., 1993), the reduction in the GnRH secretion in this study is expected. Other reports support the finding of this study. Studies suggest that exposure to high aluminium in a group of workers may disturb hypothalamus-pituitary axis and decrease TSH release (Alessio *et al*., 1989).

GnRH release was not evaluated in this study but decrease in LH and FSH concentrations can adequately reflect decreased GnRH secretion (Ganong). Decrease in the LH release will lead to the reduction in the serum concentration of testosterone as it is shown in this study. Decrease in the level of testosterone will eventually be reflected on the peripheral sex organs functions including vas deferens, epididymis and testis.

In this study the spermatozoid counts were decreased in aluminium treated animals. Spermatozoid life cycle is highly dependent on testosterone (Ganong) and taking in account the reduction of serum testosterone levels of the aluminium treated animals, this reduction is expected. It should be noted that a report has shown that aluminium can directly deteriorate rabbit sperm motility and viability (Yousef *et al*., 2007). Since in this study aluminium was applied centrally, the direct effect of the element on spermentozoid counts.

In this study the body weight of the animals receiving aluminium was less at the end of the experiment period. It appears that central injection of the metal has reduced the food intake leading to reduced body weight. Food consumption was not measured in the study, but Muller *et al.* (1992) have shown that aluminium administration (IP) can reduce the food consumption.

Since any of the parameters were not different in the groups receiving the ACSF pH 7.4 or 3.4, it appears that the effects seen in the study is solely due to the element.

CONCLUSION

The results of this study confirms the adverse effects of aluminium which should be considered on the health aspects of the individuals who have high exposure to the elements. The results also indicate that toxic effect of aluminium can be exerted via central system.

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