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Hexavalent Chromium Toxicity in Pituitary and Thyroid Glands

TARIQ MAHMOOD, IRFAN ZIA QURESHI*, MUHAMMAD SAJID NADEEM AND MUMTAZ AHMED KHAN

Laboratory of Animal Physiology, Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad. 45320, Pakistan (TM, IZQ), Department of Zoology, University of Arid Agriculture Rawalpindi, Pakistan (MSN) and Histopathologist, CDA Hospital, Civic Center, Melody, Islamabad (MAK)

Abstract.- Hexavalent chromium as $K_2Cr_2O_7$ (60mg/$kg_{b.w}$) was administered intraperitoneally to male Sprague Dawley rats. In the thyroid gland, chromium concentration decreased significantly ($P<0.01$) as compared to the control tissue. Serum FT_3 and FT_4 levels decreased significantly ($P<0.01$ and $P<0.001$ respectively), while serum TSH concentration increased significantly ($P< 0.01$) than the control. Histologically, in the pituitary gland, hypertrophy was evident in the anterior pituitary gland hypertrophied, the cellular density (number of cells/0.021mm^2) decreased significantly ($P<0.001$) and the cellular diameter increased significantly ($P<0.001$). In the thyroid gland, follicle number increased significantly ($P<0.001$) when compared to control. However, follicular size showed a significant decrease ($P<0.001$). The follicles were scattered and aggregated abnormally to form small groups making large interfollicular spaces due to the disruption of the connective tissue. The height of epithelial cells also increased. The present study demonstrates that chromium in hexavalent form causes both structural and functional disturbance to the pituitary and thyroid glands and is therefore potentially toxic to these tissues.

Keywords: Pituitary gland, thyroid gland, hexavalent chromium, toxicity, thyroid hormones.

INTRODUCTION

Chromium exists in two valence states in nature: hexavalent chromium (Cr VI) and trivalent chromium (Cr III). Chromium VI is commonly used in industrial chrome plating, alloys, cast iron and wood treatment and is a proven toxin. Previous studies demonstrate that chromium VI induces oxidative stress, DNA damage, apoptotic cell death and altered gene expression. Conversely, chromium III is an essential trace element of animal bodies; is known to regulate insulin function and is also required for normal protein, fat and carbohydrate metabolism (Bagchi *et al*., 2002). Its deficiency is associated with hyperglycemia which is reversed by supplementation (Vehage *et al*., 1996).

Cr VI compounds are approximately 1000-fold more cytotoxic and mutagenic than trivalent compounds in cultured diploid human fibroblasts (Biedermann and Landolph, 1990). Christian and Trenton (2003) have shown that the exposure of rats to compounds altering thyroid functions such as ammonium perchlorate in drinking water significantly increases relative thyroid weights, causes thyroid hypertrophy and hyperplasia and statistically significant differences in TSH, T_3 and T_4. Cr VI also causes reproductive toxicity of the testes in bonnet monkeys. It disrupts spermatogenesis, leading to the accumulation of prematurely released spematocytes, spermatids and uni- and multinucleate giant cells in the lumen of seminiferous tubules (Aruldhas *et al*., 2005).

Fatima *et al*. (2005) studied the effects of Cr VI on the kidneys of monkeys and showed that a single dose of $K_2Cr_2O_7$ leads to impairment in the function of renal brush border membrane. Cr VI causes allergic dermatitis, toxic and carcinogenic effects in humans and animals (Stohs and Bagchi, 1995; Kawanishi *et al.*, 2002). Acute and chronic neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity, immunotoxicity and general environmental toxicity have been extensively demonstrated (Von Burg and Liu, 1993; Barceloux, 1999). Soluble and insoluble Cr VI salts induce

* Corresponding author: e-mail address: irfan_qureshi_pk2007@yahoo.com,

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morphological and neoplastic transformation and mutagenecity in murine and human cells (Patierno *et al.*, 1988).

Effect of Cr toxicity has been extensively studied on the kidneys, liver, blood and some other tissues but there is no report as regards the toxic effects of Cr VI on the pituitary and thyroid glands. The present study was therefore designed to investigate chromium toxicity caused to pituitary and thyroid glands using rat as model system.

MATERIALS AND METHODS

Animals

Sixty adult male Sprague Dawley rats (av. body wt. 225g) were obtained from the National Institute of Health (NIH), Islamabad and maintained on a semi-synthetic rat chow and water *ad libitum* for 15 days prior experimentation. Photoperiods maintained were 12 hours light and 12 hours darkness. The animals were divided into control and experimental groups each containing 30 rats. N=25 rats from each group were used for the estimation of the metal concentration through atomic absorption spectrophotometry, while the remaining five rats from each group were utilized for histological examination. Experimental procedures were carried out according to the guidelines provided by the local Ethical Committee for Scientific Research on Animals, Department of Sciences, Quaid-i-Azam University, Islamabad.

Twenty five experimental rats were administered intraperitoneally potassium dichromate ($K_2Cr_2O_7$) at the rate of 30mg/kg body weight (b.w.). After 24 hours, the animals received another dose at the same concentration. Rats were sacrificed after a total of 48 hours. The control group of five rats maintained in parallel received 0.9% physiological saline instead of $K_2Cr_2O_7$.

Collection of blood and tissues

Pituitary and thyroid tissues and blood were obtained for the determination of chromium concentration, hormonal analysis and histological examination.

The blood was drawn directly from the left ventricle through cardiac puncture. It was allowed to stand for 1 hour at room temperature and later centrifuged at 1258xg for 15 minutes for serum preparation.

Estimation of chromium

For estimation of Cr concentration through the atomic absorption spectrophotometry (Fransion, 1981), the pituitary and thyroid glands of 25 rats were pooled into five groups, each consisting of tissues from five animals. Tissues and serum samples were pre-digested with 5 ml HNO_3 (69 % pure, Merck, Germany) for overnight at room temperature. Then they were heated on a hot plate at about 200°C for complete digestion and then filtered. Digests thus obtained were diluted to 8 ml with distilled water. The samples thus obtained were subjected to air-acetylene flame of atomic absorption spectrophotometer (Shimadzu AA-670 Japan). Peak wavelength for chromium was 375.9 nm, lamp current was 5 mA and band pass was 0.5 nm. Final metal concentration in each sample was calculated by using the formula:

$$\frac{\text{Absorbance of the sample (ppm) x 8.0 (ml)}}{\text{Weight of the tissue (0.5g)}}$$

Estimation of hormones

Serum thyroid stimulating hormone (TSH), free thyroxine (FT_4) and free triiodothyronine (FT_3) were determined using commercially available kits (Immunotech a.s.-Radiova 1-102 27 Prague 10-Czech Republic). Serum TSH, FT_4 and FT_3 were assayed using standard competitive radioimmuno-assay (RIA) procedures. Bound radioactivity was measured as cpm in a gamma counter. Mean sensitivities of the assay were, 0.4 pmol and 0.5 pmol for serum FT_4 and FT_3, respectively. Mean intra-assay coefficients of variation were equal to 3.7%, 6.7% and 6.4% for serum TSH, FT_4 and FT_3, respectively.

Histopathology and morphometry

Pituitary and thyroid glands of ten rats were processed for standard histological procedures. Six micron thick paraffin wax embedded tissues were stained with hematoxylin and eosin. Cell sizes were determined using the stage and ocular micrometers,

while the number of the pituitary cells and thyroid follicles were determined / 0.021mm^2 (5250 µm^2) of the area at 320x magnification in selected sections.

Statistical analysis

The results obtained were analyzed and compared using student's unpaired t-test through Excel software (Microsoft Windows XP, USA). P<0.05 was considered significant difference.

RESULTS

Chromium concentration

Cr concentration in the thyroid gland decreased significantly from 0.57±0.09 meq/L to 0.18±0.01 meq/L (p<0.01), whereas no significant difference was observed in the Cr content of the pituitary gland as the levels were 0.59±0.09 meq/L in control and 0.73±0.06 meq/L in the treated groups (P=0.377). However, serum Cr concentration showed a non-significant increase and the levels of chromium were 0.25±0.00 meq/L and 0.27±0.01 meq/L in control and treated groups, respectively (Table I).

Table I.- Mean Cr concentration (meq /L) in the pituitary and thyroid gland and blood of control and Cr- treated rats. Values are expressed as mean ±SE. (Sample size, n=5 in all cases)

Tissues	Control	Treated
Pituitary gland	0.59±0.09	0.73±0.06$^{n.s}$
Thyroid gland	0.57±0.09	0.18±0.01*
Blood	0.25±0.00	0.27±0.01 $^{n.s}$

*P<0.01; n.s = non-significant

Hormone concentrations

The control and Cr treated serum samples showed that the concentration of FT_4 decreased from 19.46±0.57 to 09.7±2.16 pmol/L (P<0.01). Similarly the FT_3 concentration decreased from 04.0±0.18 pmol/L to 01.89±0.29 pmol/L (P<0.001). On the contrary, serum TSH concentration increased from 4.66±0.15 to 11.77±1.96 ng/ml (P<0.01) (Table II).

Histopathological studies

Histological sections of Cr treated pars distalis showed hypertrophy of the pituitary (Fig. 1). Individual cells and their nuclei appeared slightly larger (Table III). The cells were disposed in sheets and trabeculae. The cellular density showed a significant decrease from 2441.32±77.30 to 1276.86±67.40 (P<0.001). However, the diameter of cells showed a significant increase from 8.00± 0.24 to 10.79± 0.41 (P<0.001) (Table III).

Table II.- Mean serum FT_4 (pmol/L), FT_3 (pmol/L) and TSH (ng/ml) levels in control and chromium treated rats. Values are expressed as mean ± SE. (Sample size, n=5 in all cases).

Hormones	Control	Treated
FT_4 (pmol/L)	19.46±0.57	09.72±2.16*
FT_3 (pmol/L)	04.0±0.18	01.89±0.29**
TSH (ng/ml)	4.66±0.15	11.77±1.96*

*P<0.01; **P<0.001

Table III.- Mean cellular densities and size of the cells of control and chromium treated pituitary and thyroid glands at 320x. Values expressed as Mean±SE.

Glands	Cellular densities (/0.021 mm^2)		Cellular diameter (µm)	
	Control (n=6)	Treated (n=6)	Control (n=30)	Treated (n=30)
Pituitary (Cells)	2441.32± 77.30	1276.86± 67.40***	8.00± 0.24	10.79± 0.41**
Thyroid (Follicles)	67.78± 1.64	156.51± 4.06	135.25± 8.44	69.86± 5.07***

*** = <0.001

The normal untreated rat thyroid showed large sized follicles filled with colloid and were bounded by peripheral epithelial cells. The epithelial cell height was normal and no interfollicular spaces were apparent (Fig. 2, A and B), whereas chromium treated thyroid showed increase in number of follicles when compared to control. The follicles showed a significant increase from 64.78±1.64 to 156.51±4.06 (P<0.001) but, the follicular size decreased from 135.25±8.44 to 69.86±5.07 (P<0.001) (Table III).

Necrosis was also evident (Fig. 3A). The connective tissue was dissolved as a result of collapse of the follicles (Fig. 3B). The dissolution of the connective tissue resulted in the formation of

large interfollicular spaces. A few follicles were fused (Fig. 3C) and the epithelial cell height appeared increased as compared with the control.

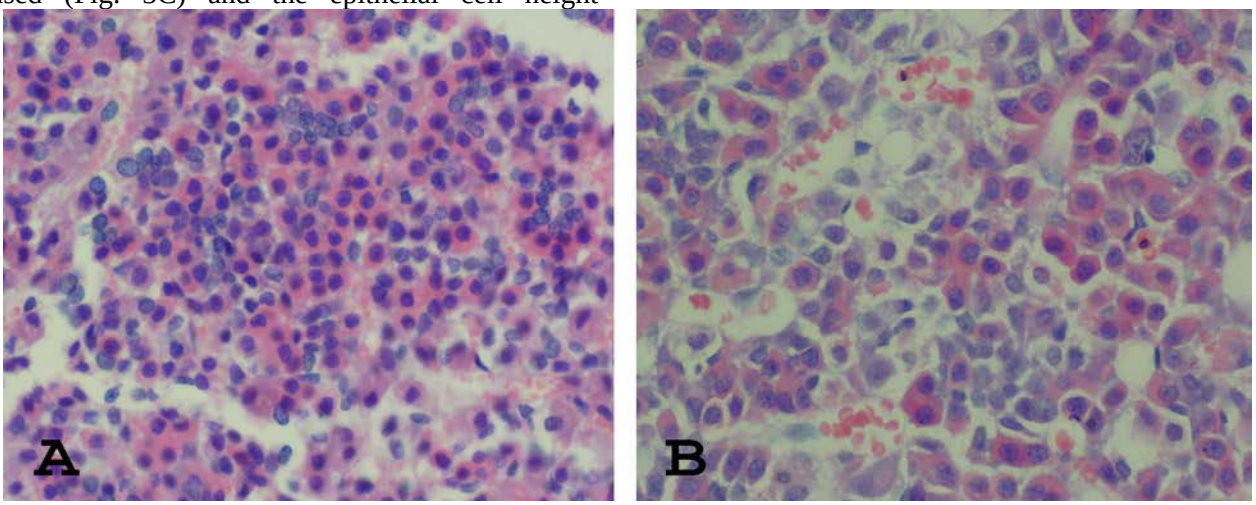

Fig. 1. Histological sections of anterior pituitary gland of rat. A, showing control pituitary gland with normal pituitary cells (1280x); B, Cr- treated anterior pituitary showing hypertrophy of the cells (1280x).

Overall appearance of tissue sections indicated an increase in the number of small blood vessels and fibro adipose tissue surrounding the thyroid. Colloidal space was reduced (Fig. 3D) (Table III).

DISCUSSION

Present investigation on rat thyroid and pituitary on acute exposure to Cr VI demonstrated noticeable physiological and structural changes most probably caused by the Cr toxicity. It appears that the pituitary gland did not accumulate Cr significantly while on the other hand thyroid gland somehow lowered its Cr burden. Since Cr is a well established trace element of the body and is involved in several physiological processes, a significant decrease of Cr concentration in the thyroid indicates additional loss of Cr by this gland. Pilat-Marcinkiewicz *et al.* (2003) showed that thyroid gland accumulates less quantity of metal when exposed to cadmium in comparison to its accumulation in the other organs, especially in the liver and kidneys. Solis-Heredia *et al*. (2000) had the similar findings that subcutaneous injections of Cr VI as $K_2Cr_2O_7$ into rats followed a dose dependent pattern of the metal and the levels of Cr were lower in the pancreas and liver but higher in the kidneys, suggesting different tissue bioavailability and accumulation pattern. Sutherland *et al.* (2000) investigated the Cr concentrations in various tissues after chronic Cr (VI) ingestion in drinking water and found that testicular Cr concentrations were elevated in the rats while in the brain, ovaries and blood, Cr concentrations were below detection limits in all exposure groups.

From the hormonal profile, it appears that Cr toxicity led quite possibly to a state of hypothyroidism as indicated by a significant increase in the serum TSH and a decrease in the serum FT_3 and FT_4 concentrations. This decreased FT_4 and FT_3 concentration in the treated animals suggest development of secondary hypothyroidism. Kobal *et al.* (2000) found that the sub lethal doses of 2,4-dichlorophenoxyacetic acid in Wistar rats significantly decreases serum T_4 and T_3 concentrations in the male groups of experimental rats. Similarly, Pilat-Marcinkiewicz *et al.* (2003) also showed that metals like cadmium decrease the T_4 levels and increase the T_3/T_4 ratio in rats with non-significant increase in serum TSH concentration. Waring *et al*. (1996) reported that plasma T_3 and T_4 concentrations increased in sub lethally Al-stressed brown trout (*Salmo trutta*). Hypertrophy of the pituitary gland and thyroid follicles hyperplasia as were shown by the tissue sections provide further evidence in favor of the

hyprothyroidism as indicated by the hormonal profile. Disorganization of the thyroid follicles and cytoarchitecture clearly indicate high level of toxicity caused by the chromium.

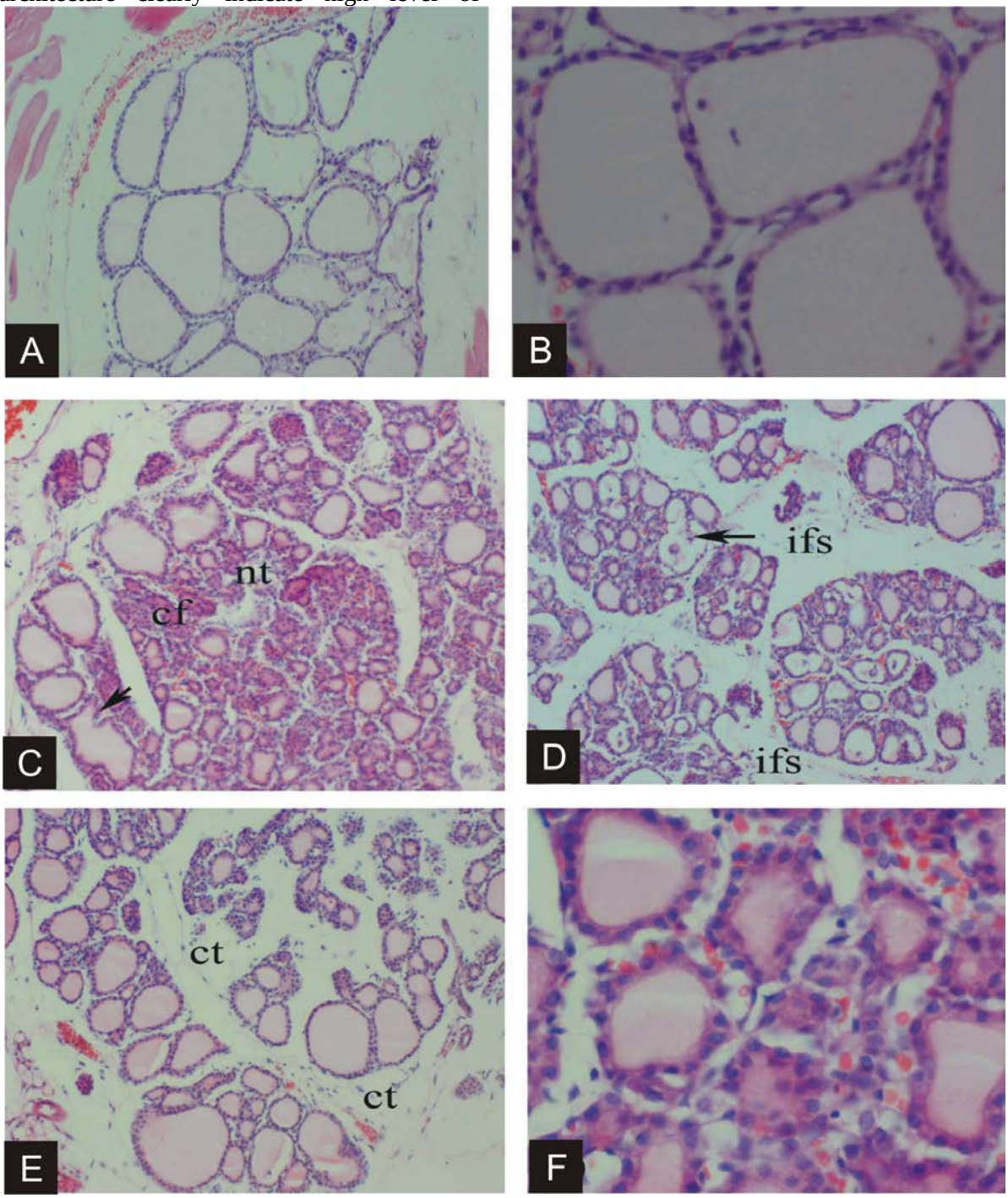

Fig. 3. Histological sections of control (A, B) and Cr- treated rat thyroid (C, D, E, F) showing normal follicles with colloid, normal epithelial cell height and no interfollicular spaces. (A. 320x; B. 1280x). C, shows follicular hyperplasia, collapsed follicles (cf), and necrosed tissue (nt). (320x); D, disorganization of the follicles and dissolution

of the connective tissue (ct) in a chromium treated thyroid (320x); E, shows large interfollicular spaces (ifs) among various groups of follicles, arrow showing fusion of follicles (320x); F, treated thyroid showing increased epithelial cell height (1280x).

There are numerous reports about the effects of heavy metals on the thyroid glands of rats and other animals. Pilat-Marcinkiewicz *et al.* (2003) demonstrated that Cd, in spite of its low accumulation in the thyroid, damaged dose-dependently the structure and function of the thyroid follicular cells. At the exposure to 5 mg Cd/L, changes occurred in their structure, but not in the function. However, the intoxication with 50 mg Cd/L led to both structural and functional damage. Similarly, Aktac and Baker (2002) investigated the effects of aluminium on the thyroid gland of rats and showed that progressive degenerative changes in the thyroid occurred which were dependent on the dose of aluminium. They further showed that some of the cells lost their nuclei and the cytoplasm. Damaged nuclei within follicle lumen and increased fibers within dispersed stroma were also apparent.

Christian and Trenton (2003) showed that the exposure of rats to compounds altering thyroid functions such as ammonium perchlorate in drinking water at 3.0 and 30 mg/kg/day significantly increases relative thyroid weights, causes thyroid hypertrophy and hyperplasia and statistically significant differences in TSH, T_3 and T_4 occurred in 30 mg/kg/day dosage groups. Furthermore, Khotimchenko *et al.* (2004) showed that **a**dministration of lead into rats resulted in reduced blood hormone levels, decreased thyroid gland weight, and impaired alterations in histomorphology. Although Cr was given intraperitonealy, it is known that Cr VI readily undergoes reduction and is converted into the Cr III oxidation state as soon as it enters into the body (Braceloux, 1999); it is not certain by which mechanism such damage to the thyroid and pituitary glands could have been caused. The observations therefore demand further investigation.

The study demonstrates that Cr VI at the rate of 60mg/kg body weight caused hypertrophy of the anterior pituitary cells with enhanced secretion of TSH, while in the thyroid gland it led to an increase in the number of follicles with disorganized structure and large interfollicular spaces that appeared among the follicles, dissolution of the connective tissue and decrease in concentrations of T_4 and T_3. We conclude that Cr VI at above dose is potentially toxic to pituitary and thyroid glands.

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