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Histopathological Effects of Hexavalent Chromium in Mouse Kidney

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All around the world several million industrial workers are potentially exposed to chromium and chromium containing compounds. These types of compounds are found in the workplace and in general environment as they are widely used in industry for welding, chrome plating, manufacture of dyes and pigments, leather tanning, and wood preserving (ATSDR 2000). Cr(VI) compounds are well recognized as human carcinogens (IARC 1990; Chiu et al. 2004; Zhitkovich 2005). Previous studies performed *in vitro* and *in vivo*, the later in both animals and humans have shown the genotoxic and carcinogenic effects of chromium compounds (Gibb et al. 2000; Bagchi et al. 2002). Cr(VI) in the form of tetrahedral chromate anion (CrO_4^{2-}) crosses cell membrane using a non specific anion carrier, the so called permease system which transports a number of anions with tetrahedral configuration such as SO_4^{2-} and PO_4^{2-} (Belagyi et al. 1999). Once inside the cell, Cr(VI) compounds are reductively metabolized by cellular reducers like glutathione, ascorbic acid or cystein to trivalent species (Gaggelli et al. 2002). This reduction is an important mechanism to inactivate Cr(VI). However, during this process a wide range of genetic lesions are generated, namely Cr-DNA adducts, DNA protein crosslinks or single strand breaks (O'Brien et al. 2003; Zhitkovich 2005).

Chromate has been established as a promoter of severe renal injury (Costa 1997). Previously, Gumbleton and Nicholls (1988) have described tubular necrosis in the inner and outer cortex of rat kidney, at a dose of 20 mg potassium dichromate/kg body weight. Subcutaneous administration of potassium dichromate into rats results in chromium accumulation mainly in the renal cortex. This accumulation is restricted to the proximal convoluted tubule, where the metal is reabsorbed and concentrated particularly in the epithelial cells.

In the present work, the effects of hexavalent chromium exposure for a 6 day period on mouse kidney were evaluated. In addition, a possible recovery study was performed after withdrawal of the treatment.

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MATERIALS AND METHODS

In this study two months old male ICR-CD1 mice purchased by Harlan Interfauna Iberica (Barcelone), and kept under laboratory controlled conditions (temperature 22±2°C, relative humidity 40-60%, 12 hour light/dark cycle) were used. Water and food were provided *ad libitum* to all animals. Animals were administrated with a daily dose of 0.3 ml of 30 mg of K_2CrO_4/kg body weight for 6 consecutive days. Potassium chromate was dissolved in 0.9% NaCl, and then subcutaneously injected into mice. Control animals were administrated for the same period with the saline solution only. Animals were divided in four groups (n=3-4) corresponding to different times of exposure until sacrifice. Mice of group I were sacrificed the day after the last administration and animals of groups II, III and IV were sacrificed 2, 4 and 6 weeks after the last injection, respectively. Control groups received an equivalent treatment. Mice were sacrificed by cervical dislocation and the kidneys were removed. Both body and left kidney weight were recorded. The left kidney was then fixed in Bouin's fluid, dehydrated, and embedded in paraffin wax. Longitudinal tissue sections 5-6 μm thick were made in a microtome (Leitz model 1512) and stained with haematoxylin and eosin (H&E). Tissue sections were also stained with Periodic Acid Schiff (PAS) and Brilliant Indocyanin 6B (IB6B), for identification of carbohydrates and protein-rich material, respectively. Each kidney slide was screened for histopathological alterations in a "blind" procedure way. The presence of granular or hyaline casts and epithelium desquamation was assessed. In each section, 40 renal tubules (20 from cortex, and 20 from medulla) were analyzed. The results were set as percentage of altered tubules. Statistical analyses of body and kidney's weights were performed using a one-way analysis of variance (ANOVA) (SigmaStat for Windows Version 3.1, SPSS Inc., USA) and the level of statistic significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Twenty-four hours after chromate injection the animals displayed apathy and reduced activity when compared with the respective controls.

The chromate administered animals from group I presented a statistically significant increase in kidney weight, and, in consequence, an increase in kidney/body weight ratio (Table 1). This increase in kidney weight is a sign of chromium nephrotoxicity. Two weeks after chromate administration and in the following weeks, kidney weight in injected animals became similar to the controls, representing a sign of recovery. Four and six weeks after chromate administration, mice presented significant lower body weight than the respective controls. A decrease in body weight was also found by Dey and co-workers (2003) in rats daily treated with CrO_3 for 28 days.

Figure 1 shows a renal section from control mice. Histological changes in the kidneys of chromate injected animals of group I revealed hyaline and granular casts and desquamation of tubular epithelium in the cortex and hyaline casts in the

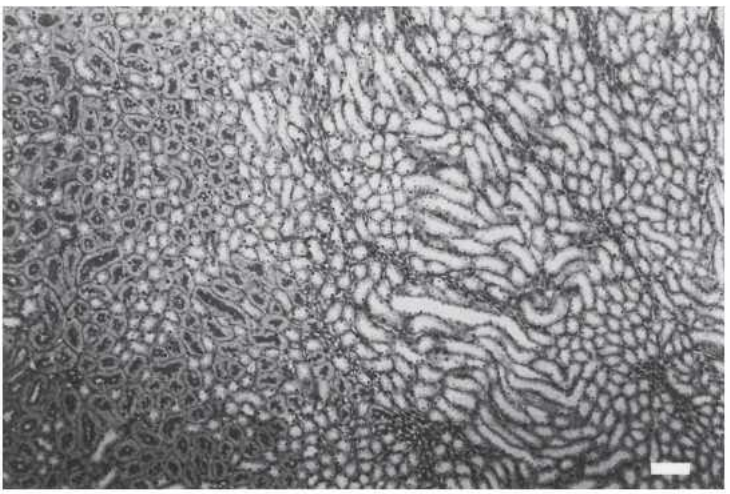

Figure 1. Histological section of renal medulla from control mice (group I). A normal structure is evidenced. PAS; x100; Bar = 80 µm.

Table 1. Body and kidney weights of mice injected with 30 mg K_2CrO_4/kg bw for 6 consecutive days.

		Body weight (g)	Kidney weight (g)	Ratio kidney/body weight (%)
Group I	Control	34.6 ± 3.0	0.27 ± 0.03	0.79 ± 0.05
	K_2CrO_4	35.0 ± 3.0	0.35 ± 0.04 *	1.01 ± 0.04 *
Group II	Control	35.0 ± 0.9	0.30 ± 0.06	0.86 ± 0.16
	K_2CrO_4	34.8 ± 0.8	0.26 ±0.01	0.74 ± 0.03
Group III	Control	39.5 ± 0.6	0.31 ± 0.05	0.77 ± 0.11
	K_2CrO_4	37.7 ± 0.2 *	0.29 ± 0.03	0.76 ± 0.07
Group IV	Control	41.2 ± 0.5	0.34 ± 0.03	0.82 ± 0.06
	K_2CrO_4	33.8 ± 1.1 *	0.27 ± 0.04	0.80 ± 0.13

Mice from groups I, II, III and IV were sacrificed 24 hours, 2, 4 and 6 weeks after potassium chromate injection. Symbol * means significant difference ($P<0.05$). n=3-4

medulla (Figures 2 and 3, Table 2). The higher number of damaged tubules (91% in the cortex and 74% in the medulla) was found 24 hours after chromium administration (Table 2). Two weeks later, some of these lesions were recovered and no granular casts were found. Although, some hyaline casts were still present in the cortex (12%) and the medulla (26%). At the end of the experiment a progressive recovery of the lesions were noted, remaining only a low number of hyaline casts (4%) in the medulla. The hyaline casts are strongly PAS positive, which means that they are rich in carbohydrate material (Figure 2). They are also positive to IB6B meaning that they are also rich in proteins (Figure 3). The

Figure 2. Histological section of renal medulla from chromate-injected mice (group I), showing the positive reaction of the hyaline casts to PAS (arrow); x100; Bar = 80 µm.

Table 2. Quantitative analysis of tubular histological damage in kidneys of mice injected with 30 mg K_2CrO_4/kg bw for 6 consecutive days.

		Renal Cortex			Renal medulla
		Hyaline casts (%)	Granular casts (%)	Epithelium desquamation (%)	Hyaline casts (%)
Group I	Control	nd	nd	nd	Nd
	K_2CrO_4	28 ± 8	37 ± 16	26 ± 14	74± 10
Group II	Control	nd	nd	nd	Nd
	K_2CrO_4	12 ± 11	nd	10 ± 10	26 ± 21
Group III	Control	nd	nd	nd	Nd
	K_2CrO_4	nd	nd	8 ± 5	18 ± 9
Group IV	Control	nd	nd	nd	Nd
	K_2CrO_4	nd	nd	nd	4 ± 3

Mice from groups I, II, III and IV were sacrificed 24 hours, 2, 4 and 6 weeks after potassium chromate injection. Values are expressed as percentage of tubules with histological damage. n= 40 renal tubules (20 from cortex, and 20 from medulla) per mouse and 3-4 mice per group. nd= not detected.

hyaline casts are a combination of protein, fat or cellular debris and are usually formed in the distal convoluted tubule and collecting duct of the kidney. The proximal convoluted tubule presented degeneration of tubular epithelial cells with granular cast formation (Figure 3), showed by the IB6B positive cellular masses present in the tubule. The granular casts are composed of granular cell debris and proteinaceous material. Their hallmark is a grainy internal structure without any

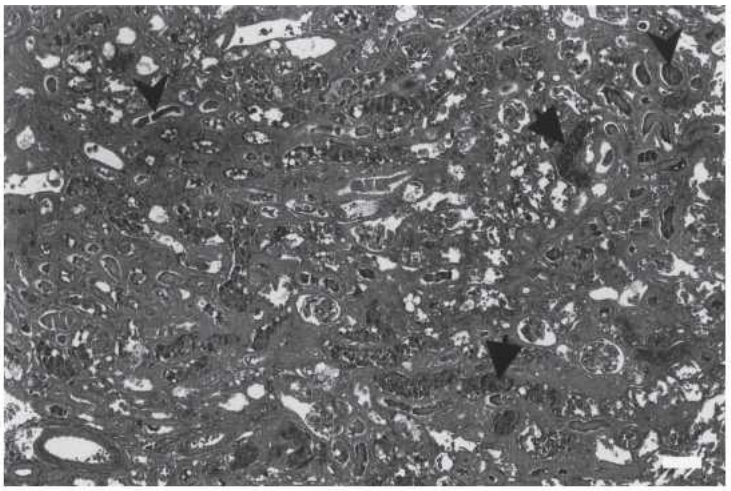

Figure 3. Histological section of renal cortex from chromate injected mice (group I), showing the positive reaction of the hyaline (arrowhead) and granular (arrow) casts to IB6B; x100; Bar = 80 μm.

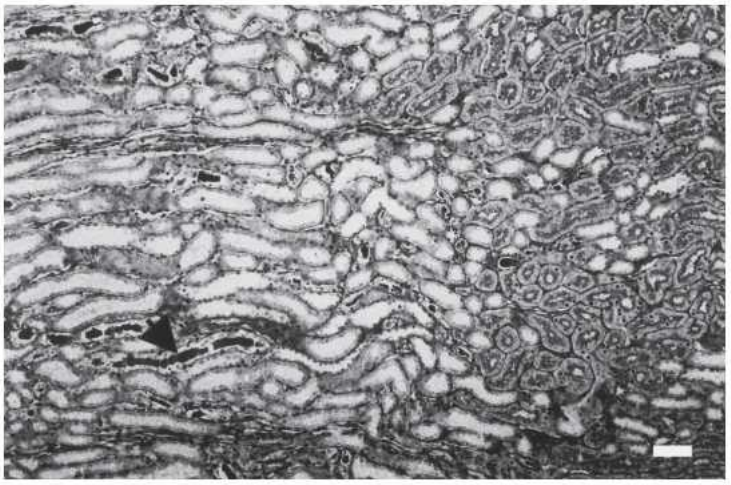

Figure 4. Histological section of renal medulla from chromate injected mice (group IV), showing the almost complete recovery with a few hyaline casts in the cortex (arrow). PAS; x100; Bar = 80 μm.

clear cellular elements. The presence of hyaline casts and cellular degeneration are the most common responses to heavy metal toxicity, namely in chromium toxicity. Laborda and co-workers (1986) described proximal tubular necrosis in rats injected intraperitoneally 3 times a week with 2 mg sodium chromate/kg body

weight. Also Wedeen and Qian (1991) reported necrosis in the convoluted portion of the proximal tubule in rats treated with both trivalent and hexavalent chromium. Potassium chromate at physiological pH exists almost entirely as chromate (CrO_4^{2-}). Chromate selective accumulation in the proximal convoluted tubule may occur due to its structural similarity to sulphate, which is primarily reabsorbed in proximal convoluted tubule (PCT) (Ruegg 1997). Depending on dose and duration of exposure, chromium is largely reabsorbed (65-90%) by the PCT (Franchini and Mutti, 1985).The depletion of cells in the Bowman's capsule may be related with the fact that in normal mature male mice the proximal convoluted tubular epithelial cells extend into Bowman's capsule surrounding the glomerulus. Since the proximal

convoluted tubule cells were highly affected these cells from Bowman's capsule suffered the same fate. In groups II, III and IV of animals some recovery was seen, mainly in the renal cortex (Figure 4). In the medulla there are still some hyaline casts, even six weeks after the last chromium administration.

The PCT has a very significant repair capacity, as seen by a histological recovery 2 weeks after chromium exposure (Figure 4). Chmielnicka and co-workers (2001) have reported a five-fold decrease in the content of chromium seven weeks after a single ^{51}Cr intraperitoneal injection. This rapid chromium excretion in the period following the administration explains the quick recovery found in chromate-injected animals from group II in the present study.

Chromium has a high affinity to the kidney. In studies using radioactive ^{51}Cr placed intratracheally in the animals indicated that chromium was selectively accumulated in the renal cortex at a 6-20 fold higher concentration than in red blood cells or liver (Weber 1983). In rats injected intraperitoneally with 2 mg K_2CrO_4/kg 6 days a week, for 45 days, the mean levels of chromium (μg Cr/g body weight) were 25.68 in the liver, 40.61 in the kidney, 7.56 in heart and 4.18 in the brain (ATSDR 2000). Of course, the route of administration affects the degree of toxicity of chromium. Kim and Na (1991) showed that dichromate injected subcutaneously to rats induced a higher degree of nephrotoxicity than when administered intraperitoneally.

In conclusion, this study showed adverse effects of potassium chromate in mice kidney after acute exposure. The main effects were cellular degeneration, mostly in the proximal convoluted tubules, and hyaline casts in the lumen of the renal tubules. These effects were seen with more intensity in the chromate injected mice 24 h after the last injection (group I). In the following weeks there was almost complete recovery. At the end of the experiment some hyaline casts were still found in the medulla.

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