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Nephrotoxicity of sodium dichromate depending on the route of administration

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Abstract. A comparison of the effects of intraperitoneal and subcutaneous routes of administration of sodium dichromate on nephrotoxicity in rats was studied. Dichromate when injected subcutaneously (SC group) produced a higher degree of nephrotoxicity than when administered intraperitoneally (IP group). It caused severe progressive proteinuria followed by polyuria and glucosuria, reaching maximum levels at 3 days after treatment in the SC group, whereas it produced mild proteinuria without glucosuria in the IP group. The dose-dependent increases in blood urea nitrogen (BUN) and creatinine concentrations, shown in the SC group, were not observed in the IP group. However, between the two groups, there were no great differences in either the urinary excretion rate of chromium or the electrophoretic patterns of urinary protein in the day 1 urine specimens. Pretreatment of phenobarbital (PB) had no remarkable effect on the dichromate-induced nephrotoxicity. In contrast, it potentiated dichromate-induced hepatotoxicity, the indices of which were the elevation in serum alanine aminotransferase (ALT) activity and hepatic lipid peroxide formation. These results suggest that the dependence of dichromate-induced nephrotoxicity on the route of administration is related to the chemical forms of chromium reaching the kidney, and the necrotizing property of dichromate results from its metabolic fate in vivo.

Key words: Sodium dichromate – Nephrotoxicity – Hepatotoxicity – Lipid peroxidation – Phenobarbital

Introduction

Acute exposure to hexavalent chromium [Cr (VI)] causes renal injury in humans and animals (Hayes 1982). Cr(VI) inhibits renal respiration and gluconeogenesis (Hirsch 1973; Berndt 1975), and causes the largest metabolic disturbance in the early period after treatment (Kim and Na 1990). However, Cr(VI) shows delayed and severe histologic changes, selectively in the convoluted portion of the proximal tubule (Baines 1965; Biber et al. 1968; Evan and Dail 1974). In addition, the effects of Cr(VI) on various renal transport processes are consistently more drastic after in vivo administration than in vitro addition (Hirsch 1973; Berndt 1975).

Cr(VI), readily taken up by cells, is subject to subsequent reduction to trivalent chromium[Cr(III)] (Connett and Wetterhahn 1983). The intracellular Cr(VI)-reduction is believed to be involved in carcinogenic effect of Cr(VI), which is not mutagenic in vitro (Tsapakos and Wetterhahn 1983), but causes DNA strand breaks and cross-links in vivo and in cultured cells (Tsapakos et al. 1981; Wedrychowski et al. 1985). Changes in cellular levels of glutathione and cytochrome P-450 result in drastic alterations of the levels and types of Cr(VI)-induced DNA lesions in chicken embryo hepatocytes (Cupo and Wetterhahn 1985). However, the relationship between the DNA lesions and the toxicity of Cr(VI) has not been clearly established.

There is little information available on the effects of intracellular Cr(VI)-reduction on renal function, even though urine is the major excretory route for parenteral Cr(III) or Cr(VI) (Sayato et al. 1980; Cavalleri et al. 1985). In this study, the correlation between the nephrotoxicity and the cellular biotransformation of Cr(VI) was investigated.

Materials and methods

Chemicals. $Na_2Cr_2O_7$ was purchased in highest purity from Merck (Darmstadt, Germany). Phenobarbital was obtained from Sigma (St Louis, MO, USA). Other chemicals were analytical grade products.

Animals and treatment. Male Sprague-Dawley rats, weighing 200 ± 10 g, were used in this study. Rats were allowed free access to standard chow and water. Sodium dichromate, dissolved in saline, was injected into rats in a volume of 2.5 ml/kg body weight intraperitoneally or subcutaneously. Control animals were given an equivalent volume of saline.

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Table 1. Dose-dependent changes in non-protein-nitrogen concentrations at 3 days after a single injection of sodium dichromate[a]

Dose (mg/kg)	BUN		Creatinine	
	i.p.	s.c.	i.p.	s.c.
0	15.7±2.7	15.1± 1.9	0.51±0.03	0.50±0.03
10	16.5±2.8	21.5± 2.9[c]	0.54±0.04	0.52±0.04
20	17.8±3.1	35.0± 6.4[c]	0.65±0.06	0.97±0.28[c]
30	23.9±3.7[c]	113.5±39.1[c]	0.66±0.07	1.86±0.55[c]
40	n.d.[b]	122.0±35.9[c]	n.d.[b]	2.12±0.75[c]

BUN and creatinine concentrations are expressed as mg per dl

[a] Values represent the mean (± SD) of five to seven rats

[b] Rats died within 2 days after intraperitoneal injection of 40 mg/kg sodium dichromate

[c] Significantly different from control (p <0.01)

Blood samples were taken from the hearts of thiopental sodium (60 mg/kg, i.p.) anesthetized rats at the requisite time.

In the experiment where attempts were made to document the onset of the nephrotoxicity, rats were placed in individual metabolism cages. One control day was allowed prior to treatment. Urine was collected in glass vessels packed in ice on a daily basis. For urinalyses, the 24-h urine specimens were centrifuged at 1000 g for 10 min.

To evaluate the effects of phenobarbital (PB), an inducer of cytochrome P-450 dependent monooxygenases, on the toxicity of sodium dichromate, PB (80 mg/kg, in saline) was injected intraperitoneally once daily for 3 days prior to sodium dichromate administration; the last PB injection was given 24 h prior to dichromate dosing. The corresponding control animals received saline.

Analytical procedures. Urinary protein was determined by the method of Pesce and Strande (1973) with bovine serum albumin as standard. Urinary glucose was determined with Asan Glucose Set^R reagent (Kyoungkido, Korea) utilizing the glucose oxidase and peroxidase reactions. Urinary excretion of protein and glucose is expressed as mg protein and glucose excreted per day to normalize variation due to polyuria, respectively. Blood urea nitrogen (BUN) and creatinine concentrations were determined in serum using the Kyokuto UN-V^R reagent (Tokyo, Japan) and Asan Creatinine Set^R reagents, respectively. Alanine aminotransferase (ALT, EC 2.6.1.2) activity in serum was measured by the procedure of Reitman and Frankel (1957), using a commercially available kit. Enzyme activities are expressed in Karmen Units per ml (U/ml). Chromium was analyzed colorimetrically according to the method of Gooderson and Salt (1968). Lipid peroxidation was quantified using thiobarbituric acid (TBA) according to the method of Ohkawa et al. (1979), as modified by Jamall and Smith (1985). Values are reported as TBA-reactive substances (TBARS) per 20 mg tissue wet weight ($A_{532} \times 10^3$/20 mg tissue, wet wt).

Electrophoresis of urinary protein. Discontinuous SDS gel electrophoresis was carried out in 15% acrylamide slab gel as described by Laemmli (1970). Usually, 25 µg dialyzed urinary protein was applied to electrophoresis. After the run, the gel was stained for proteins with Coomassie Brilliant Blue R 250. Protein molecular weight markers (14200–66000 molecular weight; Sigma Chemical Company) were used to construct a calibration curve of log molecular weight versus mobility.

Statistics. Results were analyzed using analysis of variance appropriate to the experimenal design. Significance of differences between means was tested using the Student's t-test.

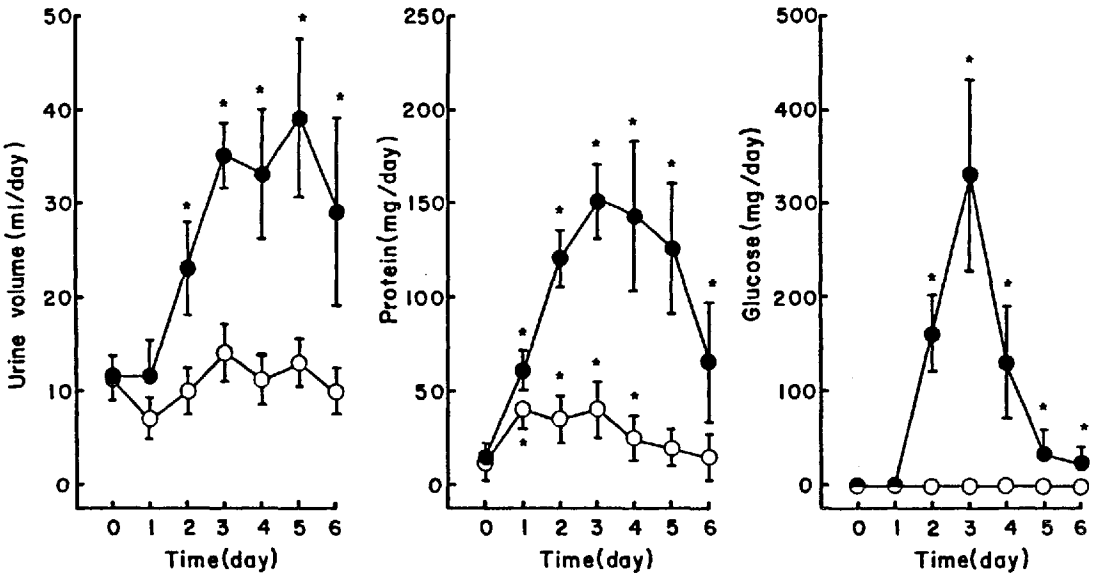

Fig. 1. Time-dependent changes in urine volume, and urinary excretion of protein and glucose after a single injection of sodium dichromate (20 mg/kg) into male rats by either intraperitoneal (○——○) or subcutaneous (●——●) route of administration. One control day was allowed prior to dichromate treatment. Daily urine samples were collected and measured. Each value represents the mean ± SD of five to seven rats.

* Significantly different from control, p <0.01

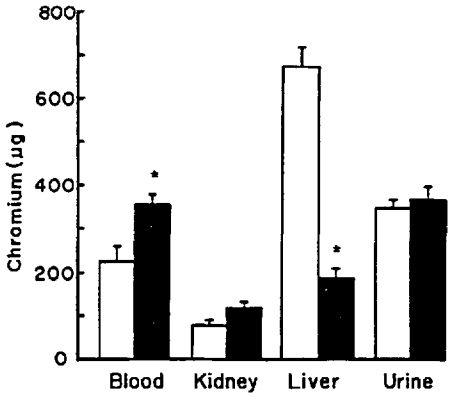

Fig. 2. Tissue distribution and urinary excretion of chromium in rats treated with sodium dichromate (20 mg/kg) intraperitoneally (□) or subcutaneously (■). Tissue chromium contents were analyzed at 30 min after treatment. Urinary excretion of chromium was determined in the day 1 urine specimens after treatment. Blood volume was assumed to be 15 ml in a 200 g rat. Results are shown as mean ± SD of seven rats. * Significantly different from the IP group, $p < 0.01$

Results

Dose-dependent changes in non-protein-nitrogen concentrations

The dose effect of dichromate on the accumulation of non-protein nitrogen (NPN) in serum, measured at 3 days after intoxication, is shown in Table 1. The NPN concentrations were progressively increased after a single injection of dichromate, reaching maximum levels at 3 days, and afterwards decreased slowly with time. However, the NPN levels did not return to normal levels at 6 days after intoxication (data not shown). Higher doses of dichromate caused significant NPN accumulations in the SC group. In contrast, the marked uremia was not produced in the IP group even with the highest dose (30 mg/kg) used here.

Time courses of dichromate-induced nephrotoxicity

Figure 1 shows the time-dependent changes in urine volume, and urinary excretion of protein and glucose after a single injection of sodium dichromate (20 mg/kg) into rats by either intraperitoneal or subcutaneous route of administration. The dose of 20 mg/kg sodium dichromate was chosen from preliminary dose-response studies which showed this to be the lowest dose which invariably produced acute renal failure. Urine volume was not affected by dichromate in the IP group during the experiment. In the SC group, urine output was significantly increased at 2 days after treatment. Polyuria, with a marked decrease in urine osmolarity, lasted for a long time.

Urinary excretion of protein was significantly increased at 1 day after treatment. However, the proteinuric effect of dichromate was less pronounced in the IP group than in the SC group, in which urinary excretion of protein was progressively increased, reaching maximum levels at 3 days, afterwards decreasing slowly with time. Increased urinary excretion of glucose, when compared to control, was not

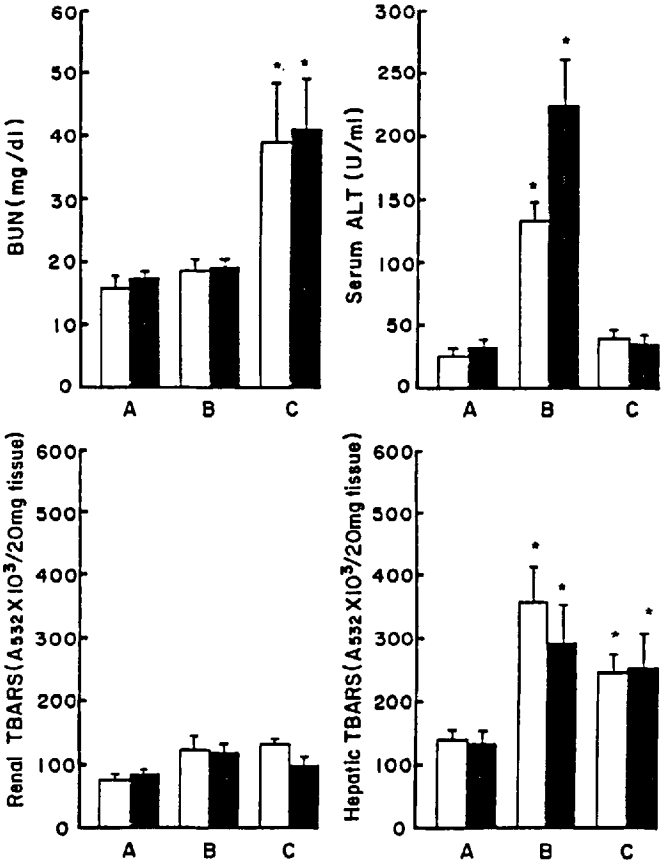

Fig. 3. Effect of phenobarbital pretreatment on dichromate-induced toxicity. Animals pretreated with PB (80 mg/kg, ■) or saline (□) intraperitoneally once daily for 3 days were sacrificed at 1 day after injection of sodium dichromate (30 mg/kg): *A*, control; *B*, intraperitoneally injected rats; *C*, subcutaneously injected rats. * $p < 0.01$ compared to control

detected in the day 1 urine specimens. Large amounts of glucose were detected in the day 2 urine when dichromate was injected subcutaneously, indicating that severe renal dysfunction occurs at this time. Urinary excretion of glucose was maximal at 3 days, but decreased rapidly thereafter. The glucosuric effect was not seen with the IP route of injection, in contrast with the effect seen after dichromate was given by the SC route, and in agreement with similar findings by Bomhard et al. (1986) with sodium chromate.

Tissue distribution and urinary excretion of chromium

Figure 2 demonstrates the patterns of tissue distribution and urinary excretion of chromium after a single administration of dichromate (20 mg/kg). Chromium was rapidly distributed in liver, kidney, and blood at 30 min after treatment. About 70% and 50% of injected chromium were distributed among three organs in the IP and SC group, respectively. The initial renal chromium contents in the SC group were consistently some higher than those in the IP group. However, there was no significant difference in the urinary excretion rate of chromium between the two groups at 1 day after treatment.

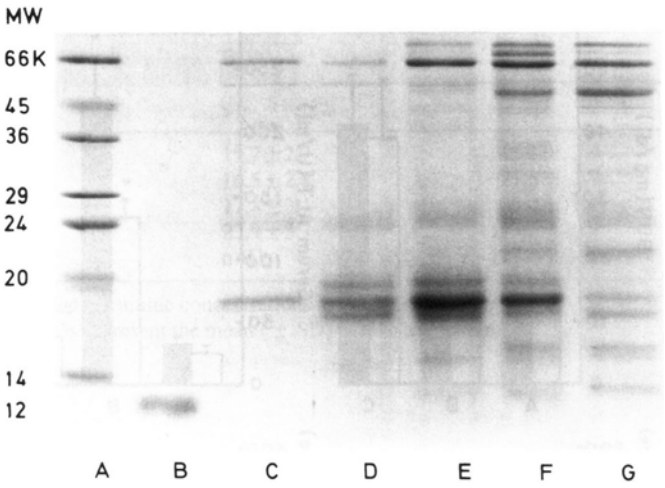

Fig. 4. Electrophoresis of urinary protein with 15% acrylamide slab gel. Urine samples, collected during day 1 after treatment, were dialyzed against deionized water at 0–4°C for 4 h. Usually, 25 μg dialyzed urinary protein was applied to electrophoresis: *A*, standard molecular weight markers; *B*, β_2-microglobulin, human; *C*, control; *D*, 14.0 mg/kg trivalent chromium s.c. (sodium dichromate was previously reduced by 10-fold excess of ascorbic acid prior to injection into the rat); *E*, 20 mg/kg $Na_2Cr_2O_7$ i.p.; *F*, 20 mg/kg $Na_2Cr_2O_7$ s.c.; *G*, 30 mg/kg $Na_2Cr_2O_7$ s.c. The gel was stained with Coomassie Brilliant Blue R-250

Effect of phenobarbital pretreatment on dichromate-induced toxicity

Figure 3 shows the PB-pretreatment effect on the nephrotoxicity and hepatotoxicity induced by dichromate. Significant increases ($p < 0.01$) in serum ALT activities and hepatic TBARS were noted in the IP group. However, dichromate produced a significant increase in hepatic TBARS without serum ALT level elevation in the SC group. PB pretreatment potentiated dichromate-induced serum ALT elevation in the IP group; however, it attenuated the increase in hepatic TBARS induced by dichromate. In contrast to hepatotoxicity, PB pretreatment had no effect on dichromate-induced nephrotoxicity in the two groups.

Electrophoresis of urinary protein

Figure 4 demonstrates the electrophoretic patterns of urinary protein in the day 1 urine specimens from dichromate-treated rats. There were no great differences in the electrophoretic patterns between the IP and SC group. The protein band having an apparent molecular weight 12000 was distinctly detected in urine from rats treated with dichromate (lanes E, F, and G). Trivalent chromium compound, obtained from reducing dichromate by 10-fold excess of ascorbic acid, did not cause renal injury.

Discussion

The results presented in this study demonstrate that the degree of dichromate-induced nephrotoxicity is dependent on the route of administration. Dichromate produced a higher degree of nephrotoxicity in the SC group than in the IP group (Fig. 1, Table 1). Liver and kidney are the critical organs for chromium accumulation (Tandon et al. 1979; Yamamoto et al. 1981). However, there were no significant differences in both the initial renal chromium contents and the urinary excretion rate of chromium between the IP and SC group (Fig. 2). Thus, it seems that chromium concentration in the kidney may not be an important factor in determining the nephrotoxic manifestation of dichromate.

Identification of the oxidation state of chromium excreted in urine seems important in elucidation of the nephrotoxic mechanism of dichromate. However, no information is available on the chemical forms, since urine has high Cr(VI)-reducing activity (data not shown). Biliary excretion of chromium is very low, and urine is the major excretory route for parenteral Cr(III) or Cr(VI) (Sayato et al. 1980; Cavalleri et al. 1985). Kidney is exposed not only to parent Cr(VI) but to a variety of hepatic and extrahepatic metabolites. Thus, it is apparent that hepatic biotransformed chromium is less toxic to renal structure and function, since there was no prominent evidence of renal injury except proteinuria in the IP group.

Cr(VI) is readily taken up by cells (Berndt 1976; Wiegand et al. 1985) and causes rapid metabolic disturbance (Kim and Na 1990); however, its nephrotoxic effects are not fully expressed at the initial time periods after treatment (Biber et al. 1968; Hirsch 1973; Evan and Dail 1974). As shown in Fig. 1, dichromate caused severe progressive nephrotoxicity, showing maximum levels at 3 days after treatment. In particular, dichromate did not cause glucosuria at 1 day after treatment even with a higher dose (30 mg/kg) which produced severe uremia and liver injury (Fig. 3). Massive glucosuria was rapidly developed only in the SC group at 2 days after treatment. However, dichromate showed the rapidity of response on 48 h-fasted rats: increased glucosuria, 185 ± 58 mg per dl urine, was observed at 1 day after subcutaneous injection of 20 mg/kg sodium dichromate. These results suggest that dichromate-induced nephrotoxicity results from its metabolic fate in vivo.

In contrast to nephrotoxicity, dichromate-induced hepatotoxicity was produced shortly after treatment, showing maximum levels at 1 day, and thereafter decreased rapidly with time (Fig. 3). Suzbstaski and Górski (1981) observed that potassium dichromate causes a rapid onset of liver injury in guinea pigs. Dichromate produced a higher degree of hepatotoxicity in the IP group than in the SC group, suggesting that the intensity of hepatotoxic effect of dichromate is greatly dependent on the initial chromium contents in the liver. In addition, dichromate caused a significant increase in lipid peroxide formation in liver injury. Yonaha et al. (1980) demonstrated that Cr(VI) causes lipid peroxide formation in rat liver microsomes. However, Cr(III) does not cause lipid peroxidation in isolated rat hepatocytes (Stacey and Klaassen 1981)

The intracellular Cr(VI) reduction is believed to be involved in carcinogenic effect of Cr(VI) (Connett and Wetterhahn 1983). The cellular levels of reducing agents such as glutathione and cytochrome P-450 are impotant in Cr(VI)-induced DNA lesions (Cupo and Wetterhahn

1985). In addition to carcinogenicity, the intracellular Cr(VI) reduction may participate in Cr(VI)-induced toxicity. This possibility is supported by the fact that pretreatment with phenobarbital, an inducer of cytochrome P-450, potentiated dichromate-induced hepatotoxicity, which was assessed by serum ALT elevation. However, it attenuated the increase in hepatic TBARS induced by dichromate. Although metabolic pathways of Cr(VI) in cells have not been fully established, this biphasic property would suggest the possibility that hepatotoxicity results from at least two different mechanisms of intracellular Cr(VI) reduction. Dichromate-induced increase in hepatic TBARS was observed in the IP and SC group. Nevertheless, a significant increase in serum ALT level was noted only in the IP group. It implies that cytochrome P-450 plays an important metabolic role only under conditions of high chromium concentration. Therefore, Cr(VI)-reducing activity of cytochrome P-450 may be the major cause of high mortality shown in the IP group after injection of 40 mg/kg sodium dichromate.

In contrast to liver injury, dichromate did not cause a significant increase in lipid peroxide formation in renal injury. PB pretreatment did not affect dichromate-induced nephrotoxicity. In addition, the types of Cr(VI)-induced DNA lesions in kidney are quite different from those of liver (Tsapakos et al. 1981). Thus, it seems that there is a great difference in metabolic pathways of Cr(VI) between the kidney and liver, which may be involved in Cr(VI)-induced toxicity.

In conclusion, the present results have shown that the dependence of dichromate-induced nephrotoxicity on the route of administration is related to the chemical forms of chromium reaching the kidney and the necrotizing property of dichromate results from its metabolic fate in vivo. The understanding of chromium metabolism in kidney should be helpful in protection and treatment of acute renal failure in chromium poisoning.

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