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Chromium increases pancreatic metallothionein in the rat

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Abstract

The ability of chromium (Cr) salts to increase metallothionein (MT) levels in rat liver, kidney and pancreas, and its relationship with the presence of toxic effects are reported here. Rats were injected subcutaneously with 0, 10, 20, 30, 40, or 50 mg $K_2Cr_2O_7$/kg and sacrificed 24 h later. Total Cr accumulation followed a dose-dependent pattern, levels in kidney being higher than those in liver or pancreas, suggesting different tissue bioavailabilities and accumulation patterns. Cr(IV) administration resulted in a tissue-specific MT induction: pancreas and liver showed five- and 3.5-fold MT increases, respectively; no increase was observed in the kidney. A positive correlation was observed between zinc and MT concentrations in liver, and between total Cr and MT concentrations in pancreas. Serum α-amylase activity showed a dose-dependent increase starting from 20 mg/kg, whereas serum glucose levels increased at doses higher than 30 mg/kg. Serum aspartate aminotransferase and alanine aminotransferase activities were increased in a dose-dependent manner, from 20 and 30 mg/kg, respectively. Our results showed that treatment with Cr(VI) can induce MT synthesis in pancreas and suggests a subsequent binding of Cr to MT. Also, pancreas is a target organ for Cr toxicity, and the usefulness of α-amylase activity as a sensitive biomarker of Cr toxicity in human exposed populations merits further study.

Keywords: Chromium; Metallothionein; Pancreas; Liver; Kidney; α-Amylase; Aspartate aminotransferase; Aspartate aminotransferase

1. Introduction

The induction of metallothionein (MT) synthesis is well known to occur in different tissues of experimental animals after exposure to various elements, such as cadmium (Cd), zinc (Zn) and arsenic (As) (Onosaka et al., 1984; Albores et al.,

Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; MT, Metallothionein.

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1992). Other factors including stress conditions (Ghoshal et al., 1998), starvation (Bremmer and Davies, 1975) and glucocorticoid treatment (Ghoshal et al., 1998; Lehman-McKeeman et al., 1988) are also known to increase its synthesis. MT is a small, cysteine-rich protein which displays high affinity for metals (Waalkes et al., 1984). MT is involved in Zn and copper (Cu) homeostasis. In addition, MT as a metal-binding protein protects against heavy metal toxicity by decreasing its bio-availability (Dunn et al., 1987). Exposure to different metals results in a quantitative variation in both tissue distribution of metals and MT accumulation. For example, the highest increase in MT content after Cd treatment was found in liver, whereas pancreatic MT was the most responsive after Zn treatment (Susuki et al., 1990). The presence and inducibility of MT in pancreas was first described by Yau and Mennear (1977) after Cd administration. There is some controversy about the responsiveness of MT induction among different pancreatic cells. Andrews et al. (1990) reported that MT mRNA levels were elevated in both endocrine and exocrine cells of rat pancreas following injection of Cd and Zn salts, whereas Minami et al. (1995) observed that MT was located immunohistochemically in the exocrine cells after Zn injection to mice. Furthermore, Kelly et al. (1996) and Onosaka and Cherian (1982) reported that pancreatic MT is a sensitive indicator of Zn status.

Chromium (Cr) is an essential micronutrient involved in insulin-related functions (Anderson, 1989), but is also a toxic element. Ghafghazi et al. (1980) showed that Cr(III) inhibits the insulin secretion probably by interfering with intracellular functions of calcium in the β-cell. MT induction by Cr has been reported in liver of mice and chick (Fleet et al., 1990; Ohta et al., 1993), but no information about Cr potential to increase extrahepatic MT levels is available, and little has been reported about its toxicity in pancreas. Therefore, the aim of the present work was to study the ability of hexavalent Cr (Cr(VI)) to increase MT levels in pancreas, liver and kidney, and its potential relationship with the presence of toxic effects.

2. Materials and methods

2.1. Animal treatments

Thirty male Wistar rats (200–250 g), equally divided into six groups, were injected subcutaneously (s.c.) with potassium dichromate ($K_2Cr_2O_7$) (0, 10, 20, 30, 40, or 50 mg/ kg body weight, equivalent to 0–24 mg Cr(VI)/kg body weight). The s.c. administration of Cr(VI) was selected for being the least aggressive. Twenty-four hours after treatment, rats were exsanguinated via cardiac puncture under deep anesthesia with ether, and organs were immediately removed for laboratory analysis. Serum was obtained for enzyme activities and glucose determinations.

2.2. MT and metal determination

MT in tissues was determined according to the silver saturation method of Scheuhammer and Cherian (1986). Silver was measured in an atomic absorption spectrophotometer (AAS) equipped with a graphite furnace (model 5100; Perkin Elmer, Norwalk, CT). For control MT analysis, an aliquot of pooled liver was analyzed in each run and less than 10% of variation coefficient was obtained. Tissue Zn, Cu and total Cr contents were determined in samples digested overnight with nitric acid, and metal concentrations were estimated by flame and graphite furnace AAS. Results were validated using the SRM1577b bovine liver from The National Institute of Standards and Technology (NIST) (Gaithersburg, MD). Variation coefficients in both determinations were lower than 10%.

2.3. Cytosolic fractionation by gel filtration

Pancreas obtained from the animals was homogenized in two volumes of 0.25 M sucrose, 1 mM dithiothreitol and 0.1 M Tris-acetate buffer, pH 8.6, containing 0.1% sodium azide. The homogenate was centrifuged at $100\,000 \times g$ for 1 h at 4°C. Three milliliters of pooled supernatant

was placed in a Sephadex G-75 chromatographic column equilibrated with the Tris-acetate, pH 8.6, buffer. Samples were eluted using the same buffer. Fractions (3 ml) were collected and MT content was monitored at 280 nm, and total Cr was quantified by AAS as already described. The Sephadex G-75 column was calibrated with rabbit liver Apo-MT I.

2.4. Biological markers of toxicity

Liver damage was evaluated by measuring serum aspartate aminotransferase (AST) (EC. 2.6.1.1) and alanine aminotransferase (ALT) (EC 2.6.1.2) activities. ALT and AST were estimated by monitoring NADH consumption (Merck Kit No. 15861 for ALT and No. 15860-9 for AST). Toxic effects on pancreas were evaluated by measuring serum α-amylase (EC 3.2.1.1) activity, as estimated by the formation of 2-chloro-4-nitrophenol (Merck Kit No. 12121), and serum glucose levels were monitored indirectly by measuring NADH production in a reaction using glucose dehydrogenase (EC 1.1.1.47) to transform β-D-glucose into D-gluconolactone (Merck Kit 12193), using a Vitalab Eclipse photometer.

2.5. Statistical analysis

Differences between control and treated groups were evaluated using a one-way analysis of variance followed by Dunnett's test.

3. Results

3.1. Metals and metallothionein levels in tissues

Total Cr concentrations in liver, kidney and pancreas after a single s.c. injection of increasing doses of Cr(VI) followed a dose-dependent pattern. At the highest dose, total Cr accumulation reached 180-, 155- and 26-fold control levels found in kidney, liver and pancreas, respectively (Table 1). Cr(VI) treatment resulted in a dose-dependent increase in pancreatic MT concentrations; the lowest effective dose was 20 mg/kg, whereas the highest dose of 50 mg/kg caused a sixfold increase, as compared with control values. In liver, an increase of three- to four-fold above control values was observed after treatment with 10 and 20 mg/kg, followed by progressive decreases thereafter (Fig. 1). No increase in kidney MT was observed at any dose (data not shown). Pancreas showed a good correlation between MT and total Cr tissue concentrations ($r = 0.96$), but not with Zn ($r = 0.68$), whereas in liver, MT correlation with Zn was $r = 0.85$ and no correlation was observed with total Cr. On the other hand, Cr(VI) did not affect Cu concentrations in the organs studied (data not shown).

The Sephadex elution profiles of proteins and total Cr and Zn content from pancreatic cytosolic fraction are shown in Fig. 2. The main protein peak and metals co-eluted at fractions 19–26, corresponding to a molecular mass range from 5

Table 1
Cr and Zn total levels in organs of rats treated with $K_2Cr_2O_7$

Dose[a]	Cr total concentration (μg/g)			Zn total concentration (μg/g)		
	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas
0	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	33.0 ± 6.0	28.0 ± 5.0	34.0 ± 15.0
10	10.0 ± 2.0*	21.0 ± 3.0*	5.0 ± 4.0	40.5 ± 5.5*	23.0 ± 7.0	30.0 ± 10.0
20	15.0 ± 5.0*	51.0 ± 18.0*	10.0 ± 7.0*	45.0 ± 7.5*	23.0 ± 7.0	32.0 ± 9.0
30	21.0 ± 4.0	66.0 ± 21.0*	14.0 ± 8.0*	39.0 ± 4.0	23.0 ± 6.0	32.0 ± 11.5
40	25.0 ± 5.0	82.0 ± 20.0	14.0 ± 4.0*	36.0 ± 6.0	23.0 ± 7.0	41.0 ± 19.0
50	31.0 ± 9.0	108.0 ± 24.0	18.0 ± 7.0*	38.0 ± 9.0	23.0 ± 6.0	45.0 ± 17.0

[a] Expressed as mg $K_2Cr_2O_7$/kg body weight. Values are the mean ± S.D. from five animals.
* Significantly different from control ($P < 0.05$).

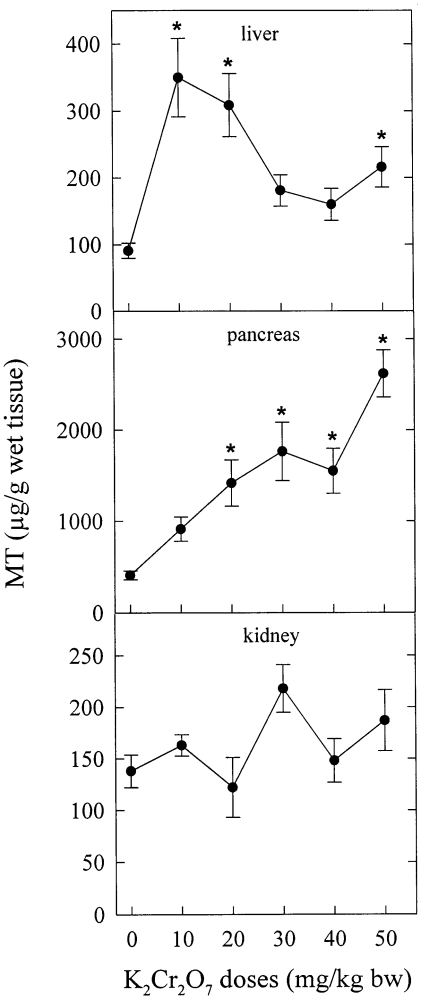

Fig. 1. Effect of Cr(VI) on MT content in liver, pancreas and kidney. MT was determined in cytosolic tissue fractions (see Section 2). Results are given as mean ± S.D.; $n = 5$; * $P < 0.05$.

to 15 kDa. This finding suggests that MT was bound to both Cr and Zn, but total Cr content was about twice as high as Zn content.

3.2. Toxicity assessment

Serum α-amylase activity showed a significant dose-dependent increase, starting from 20 mg/kg. However, serum glucose levels increased significantly only at doses of 40 and 50 mg/kg (Table 2). Regarding liver damage, serum AST and ALT activities were increased in a dose-dependent manner, starting from 20 and 30 mg/kg, respectively (Table 2). Serum creatinine levels showed a dose-dependent increase beginning at 30 mg/kg (Table 2).

4. Discussion

Our results show that Cr(VI) results in the accumulation of MT in a tissue-dependent manner, since MT content in pancreas was higher than in liver and no significant increases were observed in kidney. Pancreas is also the most responsive tissue to injections of Zn salts as reported by Onosaka and Cherian (1982) and Susuki et al. (1990). The largest relative MT accumulation occurred in pancreas, despite the lower concentration of total Cr observed in this tissue, as compared with liver and kidney. Sephadex G-75 fractionation of pancreas cytosol revealed that all the total Cr was associated with MT. The relative responsiveness of Cr(VI)-induced MT accumulation does not appear to depend as much on the tissue accumulation of the metal as it does after treatment with Cd and Zn, with the subsequent binding of both metals to MT (Susuki et al., 1990).

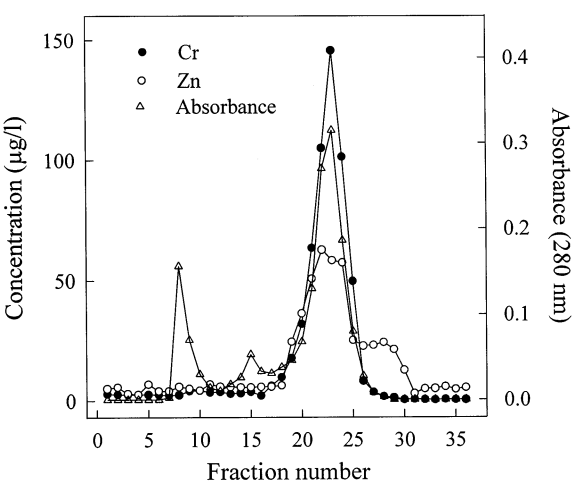

Fig. 2. Chromatographic gel filtration of pancreas cytosol. Cytosolic fraction of pancreas was loaded into a Sephadex G-75 column equilibrated with 0.1 M Tris-acetate, pH 8.6, buffer. Proteins were eluted with the same buffer. Zn and Cr co-eluted with the major cromatographic peak (280 nm absorbance).

Table 2
Effects of $K_2Cr_2O_7$ on serum creatinine, glucose, α-amylase, AST and ALT levels in rats

Dose[a]	Creatinine (mg/dl)	Serum glucose (mg/dl)	α-Amylase (U/l)	AST (U/l)	ALT (U/l)
0	0.3 ± 0.0	102.0 ± 18.0	2148 ± 183	51 ± 12	123 ± 29
10	0.3 ± 0.1	103.0 ± 24.0	2448 ± 102	55 ± 12	118 ± 13
20	0.4 ± 0.1	112.0 ± 22.0	2885 ± 123*	69 ± 15*	146 ± 22
30	0.7 ± 0.2*	107.5 ± 15.5	3206 ± 139*	81 ± 10*	188 ± 26*
40	0.8 ± 0.1*	127.0 ± 15.5*	3405 ± 102*	77 ± 7*	175 ± 31*
50	0.9 ± 0.2*	137.0 ± 20.0	3514 ± 243*	85 ± 18*	214 ± 72*

[a] Expressed as mg $K_2Cr_2O_7$/kg body weight. Values are the mean ± S.D. from five animals.
* Significantly different from control ($P < 0.05$).

Cr(VI) induction of MT could have different mechanisms depending on the organ. In pancreas, it appears to be a direct response to the metal ion, since our results show that Zn levels did not change and MT produced appears to contain Cr, whereas in the liver, MT induction seems to be sensitive to Zn status changes caused by Cr(VI) administration. This is supported by the fact that hepatic MT levels were increased at those Cr(VI) doses which resulted in hepatic Zn increases. Ohta et al. (1980) reported that Zn-MT was the main isoform induced in liver by Cr(III) exposure. Some metals may act indirectly, by displacing Zn from MT, and then Zn acts as the direct inducer or via stress associated with administration of the metal (Dunn et al., 1987). McCormick et al. (1989) reported that the metalloform of MT after Cu injection depends on the route of administration; intraperitoneal administration (i.p.) leads to a marked accumulation of hepatic Zn, whereas an intravenous injection did not change hepatic Zn and thus MT appears to contain only Cu. Albores et al. (1992) showed that MT induction after As administration resulted in Zn accumulation in liver, suggesting that this metal is the responsible inducer. Also, the induction of pancreatic MT synthesis after Cd injections is sensitive to Zn status (Onosaka et al., 1984).

In organs with a complex cellular diversity, such as pancreas, each cell type may respond differently to metals. Our results show that in pancreas, the endocrine cells were more resistant to Cr(VI) toxicity since serum glucose increased only at the highest doses, apparently for being more responsive to Cr(VI)-mediated MT increases. On the other hand, exocrine cells may not equally respond, since serum α-amylase levels increased from 20 mg/kg onwards, suggesting that exocrine cells do not induce MT or that MT did not protect them against Cr(VI) toxicity. Minami et al. (1999) reported that pancreatic MT does not provide any protection against damage caused by alloxan to endocrine cells, while Kelly et al. (1996) suggested that MT can protect pancreatic acinar cells against Zn toxicity. Andrews et al. (1990) have detected MT-mRNA in both the exocrine and endocrine cells of pancreas, but MT has been also detected only in exocrine cells (De Lisle et al., 1996; Minami et al., 1999) or in pancreatic islets (Zimny et al., 1993). The relative contribution of each pancreatic cellular type on Cr(VI)-induction of MT remains to be investigated, as well as the protective role of pancreatic MT induced after the administration of different chemicals, and its role in Cr-dependent insulin functions (McCarty, 1996).

It has been reported that MT inductive ability of Cr in liver varies according to the valence state, route of administration of the metal and the animal species. Fleet et al. (1990) showed that Cr(III) causes a greater hepatic MT accumulation than Cr(VI); and these authors also reported a two-fold increase in hepatic MT levels in chicks after an i.p. injection (150 μmol) of Cr(VI), whereas a greater effect was observed (four-fold increase) after the s.c. injection of a lower dose (68 μmol). Onosaka and Cherian (1982) also observed that the pancreatic MT levels were increased more than five-fold after a s.c. administration of Zn. Our data show that the s.c.

route of Cr(VI) administration was fully effective to induce MT in pancreas, partially effective in liver and ineffective in kidney.

MT synthesis also occurs in response to chemical stimuli, including compounds producing active oxygen free radicals, such as carbon tetrachloride (Bauman et al., 1991), acetaminophen (Wormser and Carp, 1988) and cisplatin (Bachur et al., 1978). The reduction of Cr(VI) to Cr(III) via reactive intermediates, such as reactive oxygen species, is thought to be part of its toxicity/carcinogenicity mechanism (Standeven and Wetterhahn, 1991). Thus, the generation of reactive intermediates and other factors related to Cr(VI) toxicity, such as stress and inflammation, could also play a role in the MT induction observed in this study, as suggested by Fleet et al. (1990), who indicated that the induction of MT by a number of metals is associated with an inflammatory response. MT production, then, can occur as a result of the induction of protein synthesis mediated by cytokines and hormones that are released during the initial stages of inflammation (Ghoshal et al., 1998; Huber and Cousins, 1988).

It is known that Cr(VI) is quickly reduced to the trivalent form and taken up by tissues; as a result, the kidney is regarded as a critical organ for Cr systemic toxicity (Franchini and Mutti, 1988). Thus, it seems likely that the toxicity of Cr observed in the present study impaired the ability of this organ to respond adequately to MT inducers. Kim and Na (1991b) showed a high degree of nephrotoxicity after s.c. administration of Cr(VI), which was related to the chemical form of Cr reaching the kidney. In spite of Cr(III) essentiality being closely related to pancreas, little information is available about its toxic effects on this organ. The biological active form of the glucose tolerance factor, which is an organic Cr(III) complex, is believed to function by facilitating the interaction of insulin with its cellular receptor sites (Anderson, 1989) and also by potentiating insulin action, by stabilizing its secondary structure (Govindaraju et al., 1989). Kim and Na (1991a) showed that hyperglycemia was related to both decreased serum insulin levels and direct glycogenolytic effects resultant from liver damage after Cr(VI) administration. These authors suggest that Cr(VI) alters both exocrine and endocrine pancreatic functions. Our results suggest that exocrine cells were more susceptible to Cr(VI) toxicity. An interesting finding of this study was the usefulness of serum α-amylase activity as a sensitive biomarker of acute Cr(VI) toxicity. Other authors have reported a reduced plasma α-amylase activity after the exposure to toxic compounds with no difference in glucose or insulin levels (Minami et al., 1993). The observed increased in glucose levels at Cr(VI) doses higher than 30 mg/kg might result from a decrease in serum insulin levels as proposed by Kim and Na (1991a).

In summary, the data presented in this paper demonstrated that Cr(VI) induces MT in a tissue-dependent fashion. In pancreas, MT increased according to Cr(VI) doses and was shown to bind this metal, suggesting that Cr-MT could be the prevalent metalloform present in pancreas; while in liver, MT induction was sensitive to hepatic Zn levels after Cr(VI) administration. These results suggest that Cr may be the inducer of pancreatic MT and Zn the primary inducer of hepatic MT. Our results also showed that pancreas is a target organ of Cr(VI) toxicity and serum α-amylase is a good biomarker of Cr exposure.

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