

Comparison of Susceptibility of Liver and Kidney to Lipid Peroxidation Induction by Cr(IV), Cr(V) and Cr(VI) Compounds

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(Received July 26, 1999; Accepted August 17, 1999)

Chromium(VI) compounds, potent carcinogens, are known to induce lipid peroxidation. However, it is not clear how Cr(VI) induces lipid peroxidation. It has been proposed that Cr intermediates such as Cr(IV) and Cr(V), possibly generated during the metabolic reduction of Cr(VI) to Cr(III), may be responsible for the Cr(VI)-induced DNA damage and cancer. In this article, in order to clarify the mechanism by which Cr(VI) enhances lipid peroxidation, mouse liver or kidney homogenate was incubated with Cr(III), Cr(IV), Cr(V) and Cr(VI) compounds. Our results are as follows: (i) lipids in liver homogenate are peroxidized more remarkably than those in kidney homogenate after being treated with Cr(IV), Cr(V) and Cr(VI) compounds; (ii) lipid peroxidation induction by Cr(IV) and Cr(V) compounds is more effective in both liver and kidney homogenates than that by the Cr(VI) compound; (iii) the extent of lipid peroxidation induced by the Cr compounds is significantly correlated between liver and kidney homogenates. These results suggest the possible participation of Cr(IV) and Cr(V) in Cr(VI)-enhanced lipid peroxidation.

Key words — lipid peroxidation, chromium(IV), chromium(V), chromium(VI), liver, kidney

INTRODUCTION

Lipid peroxidation is a major contributor to

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membrane damage in cells and has been implicated as a cause and effect of an extraordinary range of pathological processes associated with oxygen and free radical toxicity.¹⁾ Chromium(VI) compounds, potent carcinogens, are known to induce lipid peroxidation.²⁻⁵⁾ In contrast, Cr(III) compounds are relatively nontoxic. Lung and kidney are the main target organs of Cr(VI) toxicity; liver damage in humans and animals has not been extensively documented.^{6,7)} The kidney is much more sensitive than liver to Cr(VI)-induced cytotoxicity⁶⁾ and DNA damage.⁷⁾ However, some *in vivo* studies³⁻⁵⁾ suggest that the liver tends to be more susceptible than the kidney to Cr(VI)-enhanced lipid peroxidation. Little information is available, however, regarding the relative susceptibility of various tissues to lipid peroxidation.⁸⁾ In addition, the mechanisms of Cr(VI)-induced lipid peroxidation are not clear at present. On the other hand, it has been proposed that Cr intermediates such as Cr(IV) and Cr(V), possibly generated during the metabolic reduction of Cr(VI) to Cr(III), may be responsible for Cr(VI)-induced DNA damage and cancer.⁹⁾

This study was undertaken to investigate (i) comparison of the susceptibility of liver and kidney to lipid peroxidation induction by Cr(III), Cr(IV), Cr(V) and Cr(VI) compounds and (ii) the involvement of the metabolic conversion of Cr(VI) into Cr(V) and Cr(IV), possible Cr intermediates, in Cr(VI)-induced lipid peroxidation. By incubating mouse liver or kidney homogenate with Cr compounds, we examined the differences and correlations of the susceptibility of liver and kidney to lipid peroxidation induction by these Cr compounds.

MATERIALS AND METHODS

Chemicals — 2-Thiobarbituric acid was obtained from E. Merck (Darmstadt, Germany). Chromic nitrate ($\text{Cr}(\text{NO}_3)_3$) and potassium chromate (K_2CrO_4) purchased from Nacalai Tesque (Kyoto, Japan) were used as Cr(III) and Cr(VI) compounds, respectively. Triamminechromium tetroxide ($(\text{NH}_3)_3\text{CrO}_4$) was synthesized as a Cr(IV) compound according to the method of Riesenfeld.¹⁰⁾ Sodium bis(2-hydroxy-2-methylbutyrate)oxochromate (CrHMBA) and sodium bis(2-ethyl-2-hydroxybutyrate)oxochromate (CrEHBA) were synthesized as Cr(V) compounds according

to the method of Krumpolc and Rocek.¹¹⁾ Potassium tetraperoxochromate (K_3CrO_8) was also synthesized as another Cr(V) compound according to the method of Riesenfeld *et al.*¹²⁾

The elemental composition (%) and magnetic moment μ (B.M.) of the four compounds synthesized were determined as follows: $(NH_3)_3CrO_4$, *Anal.* Calcd for $H_9N_3O_4Cr$: H, 5.43; N, 25.15; Cr, 31.12; μ , 2.83. Found: H, 5.16; N, 24.89; Cr, 30.6; μ , 2.73. CrHMBA, *Anal.* Calcd for $C_{10}H_{16}N_8O_7Cr$: C, 37.16; H, 4.99; Cr, 16.09; μ , 1.73. Found: C, 37.65; H, 5.48; Cr, 15.5; μ , 1.72. CrEHBA, *Anal.* Calcd for $C_{12}H_{22}N_8O_8Cr$: C, 39.03; H, 6.00; Cr, 14.08; μ , 1.73. Found: C, 39.31; H, 6.08; Cr, 13.6; μ , 1.80. K_3CrO_8 , *Anal.* Calcd for K_3O_8Cr : Cr, 17.49; μ , 1.73. Found: Cr, 18.1; μ , 1.58. These data confirmed that Cr is tetravalent in $(NH_3)_3CrO_4$ and is pentavalent in CrHMBA, CrEHBA and K_3CrO_8 . Magnetic moments were measured according to the NMR method of Adams.¹³⁾

Preparation of Mouse Liver or Kidney Homogenate — Male ddY mice weighing 29–31 g were given laboratory chow (Funabashi F-2, Funabashi Farms, Chiba, Japan). Mice were killed by the scission of throat vessels. The liver or kidney was then removed, perfused, and homogenized in 10 vol of 0.10 M acetate buffer solution (pH 7.4) with a Potter-Elvehjem homogenizer and motor-driven Teflon pestle under ice cooling. The acetate buffer was selected to prevent the decomposition of Cr(IV) and Cr(V) compounds.

Incubation of Mouse Liver or Kidney Homogenate with Cr Compounds — The incubation mixture contained 1.5 ml of liver or kidney homogenate and 0.5 ml of the acetate buffer solution of Cr compound (20 mM), which gives 5 mM as a final Cr concentration; this Cr concentration was selected because lipid peroxidation levels in mouse liver and kidney homogenates reached a maximum at 10 min after incubation with 5 mM K_2CrO_4 , Cr(VI) compound, but not with Cr(VI) at lower concentrations, 0.05 mM and 0.50 mM. In the control experiment, the Cr compound was absent in the incubation mixture. Incubation was carried out in a shaking water bath at 37°C for 10 min; this incubation time, 10 min, was selected because lipid peroxidation levels in mouse liver and kidney homogenates reached a maximum at 10 min and fell at 30 min after incubation with 5 mM Cr(VI) compound.

Measurement of Thiobarbituric Acid Reactive Substances (TBARS) — Malondialdehyde (MDA), an end product of lipid peroxidation, was measured as TBARS to assess lipid peroxidation. The contents of

TBARS were measured according to the method of Masugi and Nakamura.¹⁴⁾ TBARS values were determined as nmol MDA/g tissue weight and were compared as a % of the corresponding control values,²⁾ since the lipid peroxidation levels expressed in the values relative to the control levels, but not in the absolute values, is critical to comparison of the susceptibility of liver and kidney to lipid peroxidation induction by Cr compounds when the control values are significantly different ($p < 0.05$) between liver and kidney: 263.4 ± 9.9 (mean \pm S.D.) nmol MDA/g liver ($n=4$); 392.2 ± 23.7 nmol MDA/g kidney ($n=4$).

Statistical Analysis — Results are expressed as the means \pm S.D. of four experiments. Significance between means was assessed by one-way analysis of variance (ANOVA) followed by Fisher's protected least significance difference test for multiple comparison. Comparison between the two groups was made using Student's *t*-test. The level of statistical significance was $p < 0.05$. The relationship between lipid peroxidation levels in the liver and kidney homogenates was examined by linear regression analysis.

RESULTS AND DISCUSSION

The effects of Cr compounds in various oxidation states on the lipid peroxidation of mouse liver and kidney homogenates were compared (Fig. 1). Cr(IV), Cr(V) and Cr(VI) compounds induced lipid peroxidation more strongly in the liver than in the kidney. In addition, it was found in both liver and kidney homogenates that Cr(IV) and Cr(V) compounds enhanced lipid peroxidation more efficiently than the Cr(VI) compound. Cr(III) compound failed to enhance lipid peroxidation in either of the tissues.

The lipid peroxidation levels in liver homogenates was found to correlate significantly with those in kidney homogenates after incubation with Cr(III), Cr(IV), Cr(V) and Cr(VI) compounds, and in the absence of Cr compounds (Fig. 2). The upper right sixteen data points were obtained by incubation with Cr(IV) and Cr(V) compounds, the middle four points with Cr(VI) compound, and the lower left eight points with Cr(III) compound and in the absence of Cr compounds. All data obtained could be fitted most exactly by an equation expressed as $y = 3.293x - 123.9$, where y is the lipid peroxidation level in liver homogenates and x is that in kidney homogenates.

Our data indicate that lipids in liver

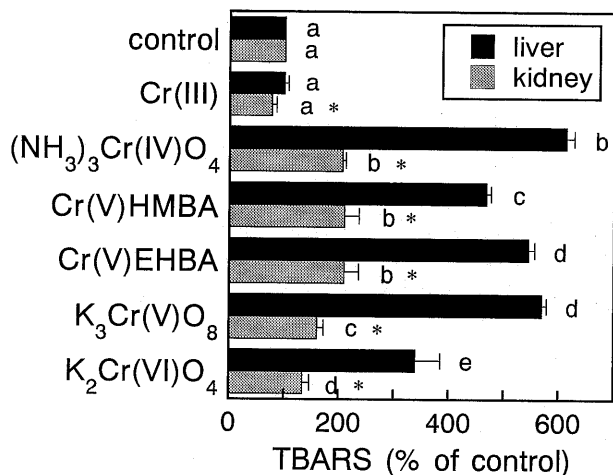


Fig. 1. Lipid Peroxidation Levels in Mouse Liver and Kidney Homogenates at 10 min after Incubation with 5 mM of Cr(III), Cr(IV), Cr(V) or Cr(VI) Compounds and in the Absence of Cr Compounds

Values expressed are means \pm S.D. ($n=4$). Original data of control groups are 263.4 ± 9.9 nmol MDA/g liver and 392.2 ± 23.7 nmol MDA/g kidney. Means not sharing a common letter in each of liver and kidney homogenates are significantly different ($p < 0.05$). *Significantly different from liver homogenate ($p < 0.05$).

homogenates are peroxidized more effectively than those in kidney homogenates after treatment with Cr(IV), Cr(V) and Cr(VI) compounds (Fig. 1). This agrees closely with the tendency for liver to be more susceptible than kidney to Cr(VI)-induced lipid peroxidation,³⁻⁵ but not with the observation that the kidney is much more sensitive than the liver to Cr(VI)-induced toxicity.^{6,7} Whether the latter discrepancy indicates that lipid peroxidation induction is not associated with toxicity by Cr compounds must be examined by further investigation.

Our result, that differences in the susceptibility of the liver and kidney to lipid peroxidation induction by Cr(IV) and Cr(V) is like those by Cr(VI) (Fig. 1), suggests (i) the similarity of the mechanism of lipid peroxidation induction by Cr(IV) and Cr(V) to that by Cr(VI), and, therefore, (ii) the possible participation of Cr intermediates, Cr(IV) and Cr(V), in Cr(VI)-enhanced lipid peroxidation. This possible role of Cr(IV) and Cr(VI) may be also supported by our result that Cr(IV) and Cr(V) strongly induce lipid peroxidation compared to Cr(VI). This is because only a part of the Cr(VI) is temporarily converted into Cr(IV) and Cr(V), possible "ultimate" toxicants for Cr(VI) toxicity⁹; concentrations of Cr(IV) and Cr(V) produced in the homogenate by a reduction of Cr(VI) are possibly much lower than those of Cr(IV) and Cr(V) compounds used in the

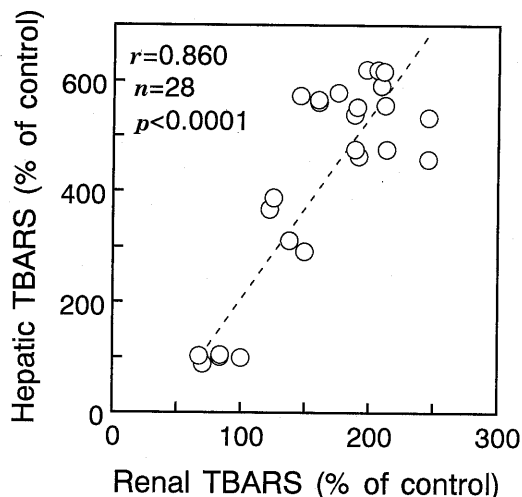


Fig. 2. Relationship between Lipid Peroxidation Levels in Mouse Liver and Kidney Homogenates at 10 min after Incubation with 5 mM of Cr(III), Cr(IV), Cr(V) or Cr(VI) Compounds and in the Absence of Cr Compounds

All data in Fig. 1 were plotted. Each point represents the values obtained from a single mouse. r : Pearson's correlation coefficient.

incubation of homogenate, 5 mM.

One possible explanation for our result of lipids in liver homogenates being peroxidized more effectively by Cr compounds than those in kidney homogenates (Fig. 1) is that the liver contains higher amounts of peroxidizable polyunsaturated fatty acids (PPUFA) compared to the kidney⁸; a labile bis-methylene hydrogen atom of PPUFA can be attacked by oxidants in lipid peroxidation initiation.¹ Liu *et al.*¹⁵ reported that the extent of lipid peroxidation of different biological organs is related to the component PPUFA, in particular, the numbers of double bonds of PPUFA. Their finding supports our explanation for the different extent of lipid peroxidation induced in liver and kidney homogenates by Cr compounds. We do not intend to exclude other explanations for the different susceptibility of liver and kidney to lipid peroxidation induction by Cr compounds.

The good correlation of lipid peroxidation induced in the liver and kidney homogenates by Cr compounds in various oxidation states (Fig. 2) suggests that the mechanisms of lipid peroxidation induction by these Cr compounds may be similar in liver and kidney homogenates.

In conclusion, (i) lipids in liver homogenate are peroxidized more remarkably than those in kidney homogenate after treatment with Cr(IV), Cr(V) and Cr(VI) compounds; (ii) lipid peroxida-

tion induction by Cr(IV) and Cr(V) compounds is more effective in both liver and kidney homogenates than that by Cr(VI) compound; (iii) the extent of lipid peroxidation induced by Cr compounds in various oxidation states is significantly correlated between liver and kidney homogenates. These results suggest the possible participation of Cr(IV) and Cr(V) in Cr(VI)-enhanced lipid peroxidation.

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