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Short Communication

Chromium (VI) potentiates the DNA adducts (O^6-methylguanine) formation of *N*-nitrosodimethylamine in rat: Implication on carcinogenic risk

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HIGHLIGHTS

- Cr(VI) synergistically enhanced the O^6-MeG formation of NDMA in rat hepatic tissues.
- Cr(VI) did not alter the CYP 2E1 enzyme activity.
- Cr(VI) exposure decreased the GSH content in rats.

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ABSTRACT

Chromium (VI) [Cr(VI)] and nitrosamines such as *N*-nitrosodimethylamine (NDMA) exist commonly in the environment. To evaluate the potential influence of Cr(VI) co-exposure on the carcinogenic risk of NDMA, Female Wistar rats were treated with various concentrations of Cr(VI) and/or NDMA via drinking water for 15 days and the DNA adducts (O^6-methylguanine, O^6-MeG) of NDMA in liver tissue was used as a bioindicator. The results showed that Cr(VI) synergistically enhanced the O^6-MeG formation, which could lead to an increase in DNA damage and carcinogenic potential. Although Cr(VI) did not alter the CYP 2E1 enzyme activity, it decreased GSH content, which would be an potential mechanism for the potentiated O^6-MeG formation by Cr(VI) co-exposure. These results would contribute to the development of quantitative risk assessment of NDMA or even for a group of nitrosamines under environmental mixture exposure.

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1. Introduction

Nitrosamines and hexavalent chromium [Cr(VI)] are toxicologically important compounds that are widely distributed in the environment. Nitrosamines are present in large quantities in cigarette smoke (Ashley et al., 2010), cured meats and smoked fish (Lijinsky, 1999), and can be endogenously formed from interaction of nitrate or nitrite with secondary or tertiary amines and amides in human stomach (Eichholzer and Gutzwiller, 1998). In recent years, nitrosamines ingested via drinking water has been found to be a new route of human exposure (Mitch and Sedlak, 2002; Ma et al., 2012). Cr(VI) is widespread in cigarette smoke and automobile emissions, and also commonly used in chemical industries (IARC, 1990). There are also concerns about Cr(VI) exposure to the general population through consumption of Cr(VI)-contaminated drinking water (Pellerin and Booker, 2000; Sharma et al., 2012). Recent studies reported that high concentrations of nitrosamines and Cr(VI) were both detected in groundwater of a district with high incidence of digestive system cancer (Ma et al., 2012; Zhang et al., 2011). Thus, human would be simultaneously exposed to the two contaminants.

Cr(VI), at physiological pH, exists predominantly as the chromate anion, and can be rapidly taken up into cells through sulfate and phosphate ion channels. Once entering into the cells, Cr(VI) can be rapidly reduced to Cr(III) by sulfhydryl groups, and glutathione (GSH) can protect against Cr(VI)-mediated toxicity (Valko et al., 2006). As one of the most important environmental carcinogen of nitrosamines, *N*-nitrosodimethylamine (NDMA) is metabolized primarily in the liver by CYP 2E1 to a methylating reactive intermediate (probably the methyldiazonium ion) which methylates cellular macromolecules (Yoo et al., 1990). GSH is also important for cellular protection against electrophiles, serving as the primary source of conjugation of NDMA-derived methylating metabolites (Limón-Pacheco and Gonsebatt, 2009). Both NDMA

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metabolites and Cr(VI) bind to GSH, suggesting that depletion of GSH by Cr(VI) might serve to decrease NDMA metabolite conjugation, and therefore increasing the potential for reaction of NDMA metabolite with DNA; however, no paper has investigated the molecular interactions of Cr(VI) and nitrosamines co-exposure.

O^6-methylguanine (O^6-MeG) is one of major methylation adducts of NDMA metabolite interacting with DNA, plays a major role in mutagenesis and carcinogenesis of NDMA (Souliotis et al., 1998). In this study, the influence of Cr(VI) on the O^6-MeG formation of NDMA in rat hepatic tissues was tested. Subsequently, CYP 2E1 enzyme activities and GSH levels were also evaluated to better understand the enhancement of Cr(VI) exposure on the O^6-MeG formation of NDMA. The results in this study are expected to contribute to the risk assessment for chemical mixtures of Cr(VI) and NDMA.

2. Materials and methods

2.1. Chemicals

Potassium dichromate, NDMA, O^6-MeG, and GSH were from Sigma (St Louis, MO, USA), and [CD_3] O^6-methylguanine (O^6-MeG-D_3) was obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). HPLC-grade acetonitrile and methanol were purchased from Fisher Chemical Co. (USA). HPLC-grade formic acid was provided by Dima Technology (USA). HCl (37%), NaOH, and *p*-nitrophenol were obtained from Beijing Chemical Co. (China). Stock solutions for all standard substances were stored at −20 °C.

2.2. Treatment of animals

Female Wistar rats, aged 6–7 weeks and weighing 170–190 g, were obtained from the Experimental Animal Tech. Co. of Weitonglihua (Beijing, China). The rats were housed in stainless steel wire-mesh cages in a temperature-controlled room on a 12-h light/dark cycle in the Laboratory Animal Center of Peking University. Rats were fed a diet prepared by the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Peking University.

The animals were divided randomly into 9 groups (n = 6/group, three per cage) and exposed to Cr(VI) and NDMA at various concentrations (Table 1). Cr(VI) and NDMA were introduced to rats via drinking water. Drinking water solutions were prepared daily. Water bottles containing Cr(VI) and NDMA were shielded from light with foil. Body weight, growth rate, fur condition, food consumption, and water consumption of all animals were monitored daily. Animals were sacrificed by cervical dislocation on the 15th day of the experiment, and their livers were sampled, weighed, and immediately frozen in liquid N_2.

Table 1
Experimental design.

Group	NDMA (mg/L)	Cr(VI) (mg/L)
1	0	0
2	0	5
3	0	20
4	0.5	0
5	0.5	5
6	0.5	20
7	1.5	0
8	1.5	5
9	1.5	20

2.3. Determination of O^6-MeG in liver tissue

DNA was isolated from liver tissue according to the method reported previously (Peterson and Hecht, 1991). The analysis of O^6-MeG followed the method as previously described (Upadhyaya et al., 2009). Briefly, each DNA sample spiked with O^6-MeG-D_3 was dissolved in 1 ml of HCl (0.1 N) and heated at 80 °C for 30 min, cooled, and neutralized with 1 N NaOH to pH 7.0. A portion of the hydrolysate (50 μL) was reserved for the determination of guanine concentration, and the rest was extracted using Strata-X cartridge (Phenomenex, Torrance, CA) which was preconditioned with 6 mL of methanol and 6 mL of ultrapure water. The cartridge was eluted with 6 mL of methanol, and the extract was evaporated to dryness under a gentle stream of nitrogen and dissolved in 0.5 mL NH_4OAc (2%).

Chromatographic analysis was carried out on an ACQUITY ultraperformance liquid chromatography (UPLC) BEH C_8 column (2.1 × 100 mm, 1.7 μm, Waters) using a Waters ACQUITY UPLC system. The column was maintained at 40 °C at a flow rate of 0.2 mL/min and isocratically eluted with 88% NH_4OAc (25 mM) and a 12% mixture of methanol and acetonitrile (75:25). The injection volume was set to 5 μL. Mass spectrometry was performed using a Waters Premier XE detector, which was operated with electrospray ionization (ESI) in the positive ion mode. The MS parameters were as follows: capillary voltage, 3.6 kV; source temperature, 110 °C; desolvation temperature, 350 °C; source gas flow, 50 L/h; and desolvation gas flow, 800 L/h. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode with the ion transitions m/z 166.0 > 149.1 with a collision energy of 19 eV for O^6-MeG and m/z 169.0 > 152.1 (40 eV) for O^6-MeG-D_3. The amount of guanine in each sample was determined by high-performance liquid chromatography according to a method reported previously (Reh et al., 2000), and adduct levels were expressed as μmol/mol guanine (μmol/mol G).

2.4. CYP 2E1 enzyme activity assay

The livers were excised, weighed and homogenized in pH 7.4 sodium phosphate buffer with 10% glycerol, and then centrifuged at 20000 g for 20 min. The supernatant was stored at −80 °C. CYP 2E1 enzyme activity was determined by measuring the hydroxylation of *p*-nitrophenol according to the procedure described previously (Koop, 1986).

2.5. Determination of GSH levels

The hepatic GSH content was determined by the method reported previously (Norris et al., 2001) and was expressed as mg/g protein. The livers were excised, weighed and homogenized in 1 mL of methanol which was kept on ice. The homogenate was then centrifuged at 800 g for 5 min. A 100 μL sample of the supernatant was evaporated to dryness under a gentle stream of nitrogen gas at room temperature and dissolved in 1.5 mL of ultrapure water.

Chromatographic analysis of GSH was carried out on an ACQUITY ultraperformance liquid chromatography (UPLC) BEH C_{18} column (2.1 × 100 mm, 1.7 μm, Waters) at a flow rate of 0.3 mL min^{-1} at 40 °C, using a Waters ACQUITY UPLC system. Mobile phase A was methanol, and mobile phase B was 0.1% formic acid in ultrapure water. The gradient (with respect to mobile phase A) was as follows: 0–3 min, 2–10% A; 3–6 min, 10% A; 6–8 min, re-equilibration with 2% A. The injection volume was set to 5 μL. Mass spectrometry was performed using a Waters Premier XE detector, which was operated with electrospray ionization (ESI) in the positive ion mode. Quantitative analysis was performed in the MRM mode with the ion transitions m/z 308.1 > 162.0 with a

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