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Responses of glutamate cysteine ligase and glutathione to oxidants in deer mice (*Peromyscus maniculatus*)

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ABSTRACT

Sensitivities of a wildlife species, deer mice, to oxidants were evaluated. A single dose (1589 mg/kg body weight by intraperitoneal injection) of carbon tetrachloride, a typical hepatotoxicant, caused changes in GCL activity and GSH content in multiple organs of deer mice. Hepatic GCL activity and GSH content were depleted substantially ($P<0.01$), renal GCL activity increased ($P<0.05$). Blood, brain and heart GCL activities increased ($P<0.05$), whereas GSH contents decreased significantly. Deer mice were exposed to Pb, or Pb together with Cu and Zn via drinking water for 4 weeks. GCL activities were not significantly affected by treatments. GSH contents were increased significantly by Pb alone, Pb with medium and high concentrations of Cu and Zn. Effects of multi-metal-contaminated soil were investigated via lactational, juvenile and lifelong exposure to feed supplemented with soils. Metal-contaminated soils did not lead to significant effects in pups via lactation, 50-day exposure altered GSH content marginally, while 100-day exposure resulted in marked GCL activity depletion. After 100-day exposure, GCL activities of the medium soil-, high soil- and Pb-treated deer mice were only 53%, 40% and 46% of the control, respectively ($P<0.0001$).

1. Introduction

Glutathione (GSH), a tripeptide composed of L-glutamate, L-cysteine and glycine, plays vital roles in biochemical and physiological functions by maintaining cellular reduction–oxidation (redox) status and protecting cells against oxidative stress. The protective capacity of GSH is due to the sulfhydryl cysteine moiety. The sulfhydryl group can non-enzymatically bind to electrophilic atoms of reactive oxygen species (ROS), or be involved in the enzymatic detoxification reaction for ROS as a cofactor for glutathione peroxidase (GPx, EC 1.11.1.9) and glutathione S-transferase (GST, EC 2.5.1.18). Through which, GSH can protect tissues such as liver and kidney from acute and chronic toxic effects (Bains and Shaw, 1997; Butterfield et al., 2002; Dickinson and Forman, 2002b; Noctor et al., 2002). Serving as an antioxidant, GSH requires continuous restoration due to its consumption. *De novo* biosynthesis is the predominant pathway for the maintenance of sufficient GSH content. Glutamate cysteine ligase (GCL, EC 6.3.2.2), which catalyzes the formation of gamma-glutamylcysteine (GC) from L-glutamate and L-cysteine, is the rate-limiting enzyme (Chen et al., 2005; Deneke and Fanburg, 1989; Dickinson and Forman, 2002a, b; Fraser et al., 2003; Hamilton et al., 2003; Janowiak et al., 2006; Soltaninassab et al., 2000; Sun et al., 1996; Toroser et al., 2006; Tsuchiya et al., 1995).

Deer mice (*Peromyscus maniculatus*) were chosen based on their nearly ubiquitous presence on contaminated sites in North America. Chemical-induced oxidative stress in wild small mammals remains a neglected subject in toxicology, the knowledge about the sensitivity of deer mice to oxidants is completely lacking. The sensitivity of deer mice to an oxidant, carbon tetrachloride (CCl_4), was explored to compare with the responses in previously studied species. CCl_4 is a prototypic centrilobular hepatotoxicant, it is a frequently used chemical to experimentally induce hepatic oxidative stress (Campo et al., 2004; Elsisi et al., 1993; Guven et al., 2003; Hartley et al., 1999; Kucharska et al., 2004; Mak and Ko, 1997; Morrow et al., 1992; Valcheva-Kuzmanova et al., 2004). CCl_4 can be reduced to trichloromethyl radical ($^{\bullet}CCl_3$) via catalysis by cytochrome P450 2E1. The trichloromethyl radical can react with molecular oxygen to form trichloromethylperoxy radical ($^{\bullet}OOCCl_3$). These radicals abstract hydrogen from membrane fatty acids, initiating and propagating lipid peroxidation. Trichloromethyl radicals may also be metabolized to phosgene ($COCl_2$), the latter is very reactive and can easily bind to cellular macromolecules due to the partial-positive-charged carbon in the molecule (Burk et al., 1984; Campo et al., 2004; Corongiu et al., 1983). Following exposure to CCl_4, GSH may be depleted as it protects against ROS produced in the metabolic process, which has been well demonstrated in laboratory rodent species (Campo et al., 2004; Valcheva-Kuzmanova et al., 2004).

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Limited reports, however, have been found to evaluate GCL activity, the predominant factor of GSH biosynthesis. Additionally, almost all studies focused on the hepatic effects of CCl_4, with little attention paid to effects in other organs. The assessment of GCL activity, as well as GSH content, in multiple tissues may better predict the overall severity of the oxidative stress and antioxidant capacity in whole organisms.

Lead (Pb) is a serious threat to human and wildlife health due to its worldwide distribution and persistence in the environment. Pb causes oxidative stress *in vivo* in both humans and experimental animals. A number of mechanisms for Pb-induced oxidative stress have been identified (Gurer et al., 1999; Hermeslima et al., 1991). Pb has a very high affinity for sulfhydryl groups of compounds such as GSH and cysteine. GSH, the most abundant non-protein thiol in cells, binds to Pb cations through its thiol group, giving rise to thermodynamically stable GS–metal complexes that can be eliminated. As a physiological defense against metal cytotoxicity, GSH content decreases with complex and excretion of Pb (Aykin-Burns et al., 2003; Butterfield et al., 2002; Canesi et al., 1998). Numerous studies have shown that both acute and subchronic Pb exposures cause GSH alterations in a variety of biological systems. In laboratory rats and mice, through drinking water, diet or intraperitoneal injection, Pb results in GSH changes in liver, kidney, brain and erythrocytes (Flora et al., 2003; Gurer et al., 1999; Hsu, 1981; Nakagawa, 1989). However, little is known about the effects of Pb on GCL. Copper (Cu) and zinc (Zn), two of the most abundant trace elements found in the human body, are intricately involved in the biochemistry of redox reactions. Studies have reported that Zn protects organisms from Pb-induced oxidative damage in rats, although Zn is a redox inert metal and does not participate in redox reactions itself (Kulikowska-Karpinska and Moniuszko-Jakoniuk, 2001; Powell, 2000; Valko et al., 2005). Differently, Cu accelerates oxidative stress by participating in the generation of ROS and catalyzing lipid peroxidation (Hsu, 1981; Stohs and Bagchi, 1995). In the meanwhile, Cu is a cofactor for superoxide dismutase (SOD, EC 1.15.1.1) (Chan et al., 1982; Klotz et al., 2003; Stohs and Bagchi, 1995). Despite the fact that it is common that these metals coexist in the field, studies on this topic are few and the effects of Cu combined with Zn on Pb-induced effects are unknown. In this study, the effects of Pb and the roles that Cu and Zn might play in altering the oxidative effects resulting from Pb exposure were evaluated. The doses in this design were based on the real situations of a contaminated site (The Anaconda Smelter Superfund site, Deer Lodge County, MT, USA), making the observed results relevant to potential effects on animal health in the field.

The Anaconda Smelter Superfund Site is contaminated with metals from nearly 100 years of milling and smelting operations for metal extraction. The metal extraction processes discharged metals into air, soils, and water, leaving more than 100 square miles of soil contaminated with a mixture of contaminants of concern (COCs) by the time the smelter closed in the 1980s. COCs include arsenic (As), cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn). A large number of *in vivo* and *in vitro* studies have demonstrated alterations in antioxidant defense systems caused by Pb (Daggett et al., 1998; Ding et al., 2000; El-Missiry, 2000; Farmand et al., 2005; Hsu, 1981; Nakagawa, 1989; Sandhir and Gill, 1995; Vaziri, 2002), Cu (Chan et al., 1982), Zn (Powell, 2000), As (Guha, 2005; Shi et al., 2004) and Cd (Alvarez et al., 2004; Roels et al., 1975). The interactions between these metals in bioavailability and effects were reported as well (Kulikowska-Karpinska and Moniuszko-Jakoniuk, 2001; Mylroie et al., 1986; Quinlan et al., 1988). Nevertheless, *in vivo* studies on the oxidative stress induced by metal mixtures are limited and studies in rodent wildlife species are completely lacking.

The study, for the first time, tested the responses of GCL activity to CCl_4, evaluated chemical-induced oxidative stress in a rodent wildlife species inhabiting the site, and provided background data for risk assessment and remediation decision-making. The metal doses in this design, originating from soil mixed in the feed, were based on the actual conditions on the sites, making the observed results highly useful for anticipating animal health in the field.

2. Materials and methods

2.1. Reagents

Chemicals used in the study were purchased from Sigma Co. (St. Louis, MO, USA) except that sodium hydroxide was obtained from Fisher Scientific International Inc. (Fair Lawn, NJ, USA). A protein assay kit (Protein Assay Kit II) was purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA).

2.2. Soil samples

Surficial (0–2 cm) soils were collected from areas on and surrounding the Anaconda Superfund site, 3 types of contaminated soils from varying subsites were sampled. They were contaminated with low, medium and high (relative terms) concentrations of COCs from aerial deposition and simply named low, medium and high soils (Table 1). All soil samples were sieved to less than 250 μm (diameter), the upper limit on particle sizes that are likely to adhere to feed or pelt and may be ingested with feed or by grooming. Soil samples were mixed into the feed at 3% (g/g), to mimic realistic environmental conditions. Pb was used as a reference, 3% of 200 μg/g of Pb was added into the feed, obtaining feed with a final Pb concentration of 6 μg/g.

2.3. Animal treatment

All procedures for animal use were approved by the Texas Tech University Animal Care and Use Committee and followed the NIH Guide for the Care and Use of Laboratory Animals. A deer mouse colony (*Peromyscus maniculatus bardii*) was established using deer mice obtained from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC, USA). Deer mice were housed in rodent boxes in a room with controlled humidity (40–60%) and temperature (18–25 °C), and maintained on a 12:12 light–dark cycle. Deer mice were allowed *ad libitum* access to drinking water and the rodent diet. Deer mice in CCl_4 dosing study received the rodent standard diet, which is a natural mixture of soybean, corn, oat, alfalfa and wheat meal (Prolab RMH 2500, PMI Nutrition International, Brentwood, MO, USA). Deer mice in metal dosing studies received a purified, casein-based defined research diet, containing 29 μg/g Zn and 6 μg/g Cu (D10001-AIN 76, Research Diets, New Brunswick, NJ, USA). All mice were allowed to acclimate to the environment for 2 weeks prior to the initiation of the treatment. Animals were dissected within 90 min of one another to lessen circadian effects on GCL activity (White et al., 1987), GSH content (Davies et al., 1983; Farooqui and Ahmed, 1984; Jaeschke and Wendel, 1985; White et al., 1987), and gamma-glutamyltransferase (GGT, EC 2.3.2.2) activity (Lin and Chen, 1997).

Ten female deer mice with age of 7–8 weeks and a mean ± SD weight of 18.6 ± 1.4 g, were randomly allocated into two groups of five each. CCl_4 was administered by intraperitoneal (IP) injection at a dose of 1.0 ml/kg body weight (equivalent to 1589 mg/kg BW). CCl_4 was mixed in two volumes of corn oil. Control mice received the same volume of corn oil by IP injection. All injection solutions were filter-sterilized prior to injection. All animals were euthanized by carbon dioxide asphyxiation 24 h post-injection. Whole blood was collected via cardiac puncture into heparinized tubes. Liver, kidney, brain and heart were removed, rinsed in ice cold 0.9% sodium chloride, blotted and weighed. The brain was dissected sagittally, an entire half was used to avoid the influence of non-homogenous distribution of GCL activity and GSH content in brain (Flora et al., 2003; Kang et al., 1999; Sandhir et al., 1994; Struzynska et al., 2002). All tissues were flash frozen on dry ice then stored at −80 °C until analysis.

Table 1
Concentrations of COCs in soils (μg/g).

Treatment	Pb	As	Cd	Cu	Zn
Control	6.6	5.6	0.3	4.2	19.5
Low	134.5	122.4	2.5	308.6	216.9
Medium	616.7	331.2	9.6	1201.4	444.7
High	1480.1	969.6	32.0	2975.8	2435.3