Sexualdimorphism of cadmium-induced toxicity in rats: involvement of sex hormones

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AbstractThe toxic effect of cadmium varies with sex in experimental animals. Previous studies have demonstrated that pretreatment of male Fischer 344 (F344) rats with the female sex hormone progesterone markedly enhances the susceptibility to cadmium, suggesting a role for progesterone in the sexual dimorphism of cadmium toxicity. In the present study, weattempted to furtherelucidate the mechanism for sex differencesin cadmium-induced toxicity in F344 rats. A single exposure to cadmium (5.0 mg Cd/kg, s.c.) was lethal in 10/10 (100 %) female compared to 6/10 (60 %) male rats. Using a lower dose of cadmium (3.0 mg Cd/kg), circulating alanine aminotransferase (ALT) activity, indicative of hepatotoxicity, was highly elevated in the cadmium treated females but not in males. However, no gender-based differences occurred in the hepatic cadmium accumulation, metallothionein (MT) or glutathione (GSH) levels. When cadmium (5.0 mg Cd/kg) was administered to young rats at 5 weeks of age, the sex-related difference in lethalitywas minimal. Furthermore, although ovariectomyblocked cadmium-induced lethality, the lethal effectsof the metal were restored by pretreatment with progesterone (40 mg/kg, s.c., 7 consecutive days) or β-estradiol (200 μg/kg, s.c., 7 consecutive days) to ovariectomized rats. These results provide further evidence that female sex hormones such as progesterone and β-estradiolare involved in the sexual dimorphism of cadmium toxicity in rats.

KeywordsCadmium toxicity•Sex difference•Progesterone•β-Estradiol •Rat

Introduction

A nonessential transitionmetal, cadmium is an important industrial and environmental pollutant. This heavy metal mainly distributes to the liver and kidney in humans and animals (Waalkes2003;Nordberg 2009). In rodents, parenteral administration of cadmium causes a rapid accumulation of cadmium in the liver and, at sufficient doses, induces severe hepatic damage in the form of hepatocellular necrosis (Kuester et al. 2002). The hepatic effects of cadmium are thought to be the primary cause of death from acute exposures (Dudley et al. 1982). On the other hand, chronic administration of cadmium commonly results in renal damage (Nordberg 2009).

It has been reported that cadmium toxicity can be modified by sex hormones including estradiol, progesterone and testosterone (Gunn et al. 1965; Maekawa and Hosoyama 1965; Wolkowoski-Tyl and Preston 1979; Shiraishi et al. 1993; Shimada et al. 1997a,b; Baker et al. 2003). For instance, Gunn et al. (1965) found that estradiol and stilbesterol protected the mouse testes against injury from cadmium. Maekawa and Hosoyama (1965) also reported that testosterone or progesterone pretreatments moderate cadmium-induced testicular damage in rats. In mice, pretreatment with testosterone had no effect on cadmium toxicity (Gunn et al. 1965), although progesterone pretreatment was quite effective in reducing cadmium toxicity (Wolkowoski-Tyl and Preston 1979). In marked contrast, previous studies have shown that pretreatment with progesterone greatly increased cadmium toxicity in vivo in rat liver (Shiraishi et al. 1993) and in vitro in rat liver cells (Shimada et al. 1997a; Baker et al. 2003). In this case, progesterone pretreatment exacerbates both cadmium-induced lethality and hepatonecrosis in vivo and cadmium cytotoxicity in vitro, despite a marked

activation of the metallothionein (MT) gene expression (Shiraishi et al. 1993; Shimada et al. 1997a). Thus, the precise nature of altered sensitivity to cadmium induced by sex hormones must be considered as ambiguous and still poorly defined.

Cadmium is also known as human carcinogen (Waalkes 2003). Recently, there is increasing evidence for cadmium having estrogenic effects (Garcia-Morales et al. 1994; Stoica et al. 2000; Johnson et al. 2003; Höfer et al. 2010). Cadmium can act like estrogens in breast cancer cells as a result of its ability to form a high-affinity complex with the hormone-binding domain of the estrogen receptor-α (Garcia-Morales et al. 1994; Stoica et al. 2000). Johnson et al. (2003) have also reported that low dose cadmium exposure to ovariectomized rats resulted in uterine hyperplasia, increased growth and development mammary glands, and induction of hormone-regulated genes. These data indicate that human cadmium exposure may be associated with hormone-related cancers including breast cancer. Indeed, the work of McElroy et al. (2006) indicate human exposure to cadmium may be a risk factor in breast cancer. In addition, there is epidemiological evidence associating cadmium exposure and endometrial cancer (Akesson et al. 2008). Thus, additional insight into gender-based differences in toxic response to cadmium is clearly warranted.

In the present study, we examined the sex differences of cadmium-induced acute toxicity and the possible roles of glutathione (GSH), MT and cadmium kineticsin male and female F344 rats. The effect of ovariectomy on cadmium-induced lethal toxicity, and the effects of sex hormones, such as progesterone andβ-estradiol on the toxicity in ovariectomizedrats were also investigated.

Materials and methods

Chemicals

Cadmium chloride ($CdCl_2$) andprogesterone were purchased from Sigma (St. Louis, MO). β-Estradioland corn oil were obtained from Wako Pure Chemicals, Tokyo, Japan.All other chemicals were of reagent grade.

Animals and treatments

Male and female F344 rats at 4 and 8 weeks of age were purchased from Japan SLC (Shizuoka, Japan). Ovariectomy of female rats was performed at 4 weeks of ageand the rats were used at 10 weeks of age. The animals were maintained on a 12-h light/dark cycle and had free access to a diet of standard laboratory chow and water. All animal experiments were undertaken in compliance with the guideline principles and procedures of Kumamoto University for the care and use of laboratory animals.

For the lethal toxicity experiment, $CdCl_2$ dissolved in saline were subcutaneously injected into 5 weeks and 10weeks of age animals at a dose of 5.0 mg/kg of body weight.When sex hormones were used, progesterone (40 mg/kg) or β-estradiol (200 μg/kg) dissolved in corn oil were given subcutaneously once a day for 7days and then $CdCl_2$ was administered concomitantly with the last injection of progesterone or β-estradiol. The survival rate (%) was assessed for 7 days after cadmium injection.

In another experiment, $CdCl_2$ were injectedsc into 10week-old animals at a dose of 3.0 mg/kg of body weight. The animals were slightly anesthetized with ether and killed by decapitation 24 h after cadmium treatment, and then immediately processed as

described below.

Assay of alanine aminotransferase (ALT) activity and blood urea nitrogen (BUN) level

To assess hepatic and renal damage, plasma ALT activity (liver) and BUN level (kidney) were assayed with commercially available kits (Wako Pure Chemicals, Osaka, Japan), and expressed as units per liter and mg per deciliter, respectively.

Tissue metal content

The contents of cadmiumin the liver and kidney were determined using atomic absorption spectrophotometry with a Shimadzu AA-6800F spectrophotometer after the tissues were digested with ultrapure nitric acid. The data was expressed as μg metal per g wet weight of tissue.

Measurements of glutathione (GSH) and metallothionein (MT)

For GSH analysis,liver and kidney were immediately homogenized in ice-cold 4% perchloric acid (1 mM EDTA). The homogenates were centrifuged at 12,000 rpm for 2 min at 4°C. The total GSH concentrations in the supernatants were determined according to the enzymatic recycling method of Tietze (1969).

The hepatic and renal MT was measured according to the method of Naganuma et al. (1987) with a slight modification using non-radioactive $HgCl_2$ (Yasutake et al. 1998). Briefly, the homogenate was treated successively with diethylmalate and 10 mM $CdCl_2$,

then heated at 95˚C for 5 min to precipitate high-molecular weight proteins. Following cooling and centrifugation, the supernatant was successively treated with 5 mM $HgCl_2$, 1 mM ovalbumin, and 12.5% TCA. After centrifugation, the supernatant was filtered through a membrane of 0.22-mm pore diameter (Ultrafree C3, Millipore) to afford Hg-MT samples. MT levels were expressed as amount of mercury bound to thionein molecules after Hg analysis. Hg levels in the final samples were analyzed by oxygen combustion-gold amalgamation method using an atomic absorption mercury analyzer MD-A (Nippon Instruments Co. Ltd., Osaka, Japan).

Statistical analysis

Data were analyzed by two-sided paired Student's *t*-test or one-way analysis of variance followed by Tukey's multiple comparison test. The difference at $P< 0.05$ was considered statistically significant.

Results

Sex difference of Cd-induced lethal toxicity

The lethal toxicity of cadmium was examined in adult male and female rats (10 weeks of age). Forty % of males survived for 7 days after subcutaneous cadmium treatment at a dose of 5.0 mg/kg body weight, whereas 100% of the females died (Fig. 1A). In fact, 90% of the females died within 24 hours of cadmium injection, a point at which 60% of the males remained alive.

Effect of Cd treatment on ALT activity and BUN level

The effects of cadmium treatment on plasma ALT activity and BUN level, indicators of hepatic and renal damage, respectively, were examined in male and female rats. When cadmium was given at a dose of 3.0 mg/kg, the ALT activity was markedly increased in females (1563 ±851 IU/l), but was not altered in males (10.7 ± 3.3 IU/l), indicating a sex difference in cadmium-induced hepatotoxicity. No significant increase occurred in the BUN levels in either the males or the females (data not shown).

Tissue Cd content

Hepatic and renal cadmium contents in male and female rats treated with cadmium at a dose of 3.0 mg/kg body weight were measured. There was no significant difference between the hepatic cadmium content in the males and females (data not shown). The renal cadmium content in the females was significantly higher than that in the males.

Effect of Cd treatment on hepatic and renal GSH and MT levels

The hepatic and renal GSH and MT levels in male and female rats treated with cadmium at a dose of 3.0 mg/kg body weight were examined (Table 1). There were no significant differencesbetween the males and females in the hepatic and renal GSH levels. On the other hand, the renal MT levelafter cadmium treatment was significantly lower in the males than in the females. There was no significant

difference between the males and females in the hepatic MT level. The lower renal MT level in males may reflect the lower renal cadmium accumulation.

Sex differences in cadmium-induced lethal toxicity in immature rats

The lethal toxicity of cadmium was furtherexamined in pre-puberial (immature) male and female rats. When cadmium (5.0 mg/kg) was administered to young rats at 5 weeks of age, sex-related difference was minimal forcadmium-induced lethality (Fig. 1B). Males showed 40% lethality (6/15) and females showed 53% (8/15)death rate over the same 7 day post-injection period.

Cadmium-incduced lethal toxicity in ovariectomized rats: effect of progesterone or β-estradiol

To examine whether female hormones were the basis of sex-related cadmium toxicity, effect of ovariectomy on cadmium-induced lethal toxicity was studied (Fig. 2). As expected, ovariectomy decreased cadmium-induced lethal toxicity of 100% (see Fig. 1A) to a level where 50% of the rats survived (Fig. 2). Furthermore, this decreased effect could be markedly reducedby the pretreatment with progesterone (40 mg/kg, s.c.) or β-estradiol (200 μg/kg, s.c.) to ovariectomized rats (Fig. 2).

Discussion

Although cadmium-induced toxicity, including lethality and hepatotoxicity, shows clear

sexual dimorphism,with females being more sensitive, the basis for phenomenon is not yet well understood. This could have important implications as it appears cadmium may target females specific tissues in development (Johnson et al. 2003) or human cancer (breast, uterus; Akesson et al. 2008; McElroy et al. 2006). The present study further examined the sex differences in cadmium-induced acute toxicity in rats. The acute lethal effects of cadmium in rodents are thought to be largely due to the production of hepatic lesions, including extensive hepatocellular necrosis (Kuester et al. 2002).The present results clearly indicate that female rats are more sensitive to cadmium-induced lethaltoxicity than male rats. Furthermore, in the present study cadmium treatment at lower dose (3.0 mg/kg)markedly increases the plasma ALT activity in females but not in males, further indicating that female rats are more sensitive to cadmium-induced hepatotoxicity than the males. Additional study showed female sex hormones directly impacted cadmium toxicity in ovariectomized animals.

Interestingly, in immature rats at 5 weeks of age, the sex differences in cadmium-induced lethal toxicity seen with adult rats (10 weeks of age) wasminimal. Inrats, circulating levels of sex hormones including progesterone are low until 5 weeks of age, and then become progressively elevated after 6 weeks of age. Thus, ovariectomyat 4 weeks of age decreased cadmium-induced lethal toxicity at 10 weeks of age. Furthermore, whenovariectomized rats were pretreated with progesterone or β-estradiol prior to cadmium treatment, the protective effect of ovariectomy was largely abolished. It has been reported that during pregnancy, a time of high circulating progesterone and estradiol, animals can be extremely sensitive to cadmium-induced toxicity (Parizek 1964, 1965; Parizek et al. 1968; Samarawickrama and Webb 1981). Recent studiesalso have demonstrated that progesterone pretreatment increases

cadmium toxicity in rats and rat liver cells (Shiraishi et al. 1993; Shimada et al. 1997a; Baker et al. 2003). In combination with the present results, these studies support the conclusion that progesterone,either given concurrentlyor as a pretreatment,directly sensitizes females to cadmium toxicity, at least within the liver. It will be of interest to test this sex hormone sensitization hypothesis in other target tissues of cadmium, such as breast and uterus (Johnson et al. 2003; Akesson et al. 2008; McElroy et al. 2006).

To elucidate whether cadmium accumulation in tissues is a basis of the sex differences on cadmium-induced toxicity, hepatic and renal cadmium contents were determined.The present results indicate there was no gender-based difference in the hepatic cadmium accumulation, the key target organ (Dudley et al. 1982; Kuester et al. 2002). Prior work indicates that progesterone pretreatment has no effect on the accumulation of hepatic cadmium in F344 rats even though the cadmium-induced lethality and hepatotoxicity are increased by the pretreatment (Shiraishi et al. 1993). In contrast, accumulation of cadmium in the liver of rats is greater in females than in males (Blazka et al. 1988; Blazka and Shaikh 1991).Blazka and Shaikh (1991) reported that in male Sprague-Dawley rats, estradiol pretreatment caused a greater accumulation of cadmium in liver. In addition, in mice, hepatic cadmium accumulation is higher in males than in females (Shaikh et al. 1993). From these results, it can be concluded that the sex difference of cadmium toxicity are not explained by altered hepatic cadmium biokineticsat this point, and cadmium-induced toxicity apparently varies with the specific species and strain (Shimada et al. 2009; Shimada et al. 2011).

Cadmium induces synthesis of, and then binds to, MT (Waalkes 2003; Klaassen et al. 2009). It is conceivable that the sensitivity to cadmium toxicity in female rats is due poorer induction of MT, resulting in lower hepatic MT level in the females than in the

males. However, in this study, hepatic MT levels after cadmium treatment showed no difference between females and males. In addition, no gender-based differencein the hepatic GSH levels, which are thought to be important in immediate cadmium detoxicationprior to MT synthesis, wereobserved. Thus, the current data showing hepatic MT and GSH levels were similar between males and females do not explain the mechanism of the sex difference on cadmium-induced toxicity.

In conclusion, the results of present study provide further evidence that female sex hormones are involved in the sexual dimorphic pattern of cadmium toxicity in rats and can, in fact, exaggerate it. Further investigations are needed to clarify the mechanism by which sex hormones enhance cadmium toxicity.

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Figure legends

Fig. 1. Sex difference in cadmium-induced lethal toxicity.Male and female rats of 10 weeks of age (A) and 5 weeks of age (B) were treated with $CdCl_2$ (5.0 mg Cd/kg, s.c.). Survival was assessed 7 days after cadmium treatment (n=10 or 15).

Fig. 2. Cadmium-incduced lethal toxicity in ovariectomized rats: effect of progesterone or β-estradiol. Ovariectomized rats were pretreated with progesterone (40 mg/kg, s.c.) or β-estradiol (200 μg/kg, s.c.) once a day for 7 days and then given $CdCl_2$ (5.0 mg/kg, s.c.). Survival was assessed 7 days after Cd treatment (n=6-10).Cadmium-induced lethal toxicity in non-ovariectomized rats was 100% (see Fig. 1).