Strain difference of cadmium-induced testicular toxicity in inbred Wistar-Imamichi and Fischer 344 rats

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Abstract Previously, we reported that Wistar-Imamichi (WI) rats are highly resistance to cadmium (Cd)-induced lethality and hepatotoxicity compared to Fischer 344 (F344) rats. Since the testes are one of the most sensitive organs to acute Cd toxicity, we examined possible strain-related differences in Cd-induced testicular toxicity between inbred WI and F344 rats. Rats were treated with a single dose of 0.5, 1.0 or 2.0 mg Cd/kg, as $CdCl_2$, sc and killed at 24 h later. Cd at doses of 1.0 and 2.0 mg/kg induced severe testicular hemorrhage, as assessed by pathologically and testis hemoglobin content, in F344 rats, but not WI rats. After Cd treatment (2.0 mg/kg), testicular Cd content was significantly lower in WI rats than in the F344 rats, indicating a toxiokinetic mechanism for the observed strain difference. Thus, the remarkable resistance to Cd-induced testicular toxicity in WI rats is associated, at least in part, with lower testicular accumulation of Cd. When zinc (Zn; 10 mg/kg, sc) was administered in combination with Cd (2.0 mg/kg) to F344 rats, the Cd-induced increase in testicular hemoglobin content, indicative of hemorrhage, was significantly reduced. Similarly, testicular Cd content was significantly decreased with Zn co-treatment compared to Cd treatment alone. Thus, it can be concluded that testicular Cd accumulation partly competes with Zn transport systems and that these systems may play an important role in the strain-related differences in Cd-induced testicular toxicity between WI and F344 rats.

Keywords Cd, Testicular toxicity, Strain difference, Rat, Zn

Introduction

Cadmium (Cd) is a very toxic nonessential metal and environmental pollutant. In humans and animals, this heavy metal distributes to various tissues such as the liver, kidney and testes, where accumulated Cd can result in tissue damage (Waalkes et al., 1992; Goering et al., 1994). The testes are extremely sensitive tissue to Cd, and the toxic effects occur despite the fact that very little Cd actually accumulates in the testes (Gunn and Gould, 1970; Shiraishi and Waalkes, 1996). The acute lesions associated with Cd exposure in the rodent testes include severe hemorrhage with edema and necrosis and this subsequently results in total disruption of the seminiferous tubules (Shiraishi and Waalkes, 1996).

Sensitivity to Cd in the testes varies widely with strain in rodents (Gunn et al., 1965; Shiraishi and Waalkes, 1996). For instance, Cd-induced lipid peroxidation, indicative of testicular toxicity, is significantly greater in the testes of Cd-sensitive NFS mice compared to Cd-resistant BALB/c mice (Abshire and Waalkes, 1993). In addition, the accumulation of Cd in the testes of Cd-resistant A/J mice has been reported to be much lower than that in the testes of Cd-sensitive 129/J mice (King et al., 1998). Several possibilities exist as to the mechanism for strain differences of Cd-induced testicular toxicity, including difference of testicular Cd-binding protein levels such as metallothionein (MT), other than a difference in testicular Cd accumulation. The basis of strain differences of Cd-induced testicular toxicity remains to be completely clarified.

We have recently found that Wistar-Imamichi (WI) rats are strongly resistance to Cd-induced lethality and hepatotoxicity compared to Fischer 344 (F344) rats (Shimada et al., 2002, 2004). In this case, the hepatic Cd accumulation in WI rats was

significantly lower than that in F344 rats (Shimada et al., 2002, 2004). Interestingly, co-administration of Zn to F344 rats markedly decreased Cd accumulation in the liver, resulting in the protection against Cd-induced lethality (Shimada et al., 2004). The strain difference in Cd accumulation between WI and F344 rats was also observed *in vitro* using liver slices, and the accumulation of Cd by liver slices of F344 rats decreased when Zn was added to the medium in combination with Cd (Shimada et al., 2008). These results suggest that the strain difference of Cd-induced hepatotoxicity between WI and F344 rats is due primarily to the differential Cd accumulation into the tissue, and that Zn transport mechanism is involved in the difference of Cd-induced hepatotoxicity in the two rat strains.

The purpose of the present study was to examine the strain difference of Cd-induced testicular toxicity in inbred WI and F344 rats under the hypothesis that accumulation into tissues plays at least a partial role. The possible role of testicular metal-binding protein metallothionein (MT) and the effect of co-administered Zn on Cd-induced testicular toxicity were also examined.

Materials and methods

Chemicals

Cadmium chloride ($CdCl_2$) was purchased from Sigma (St. Louis, MO, USA). Zinc chloride ($ZnCl_2$) was obtained from Wako Pure Chemicals (Osaka, Japan). All other chemicals were of reagent grade.

Animals and treatments

Inbred male WI (W/Iar) and F344 rats were obtained from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan) and Japan SLC (Shizuoka, Japan), respectively. 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. WI and F344 rats at 9 weeks of age were injected sc with saline or $CdCl_2$ (0.5, 1.0 and 2.0 mg/kg as Cd). To assess the effect of Zn, F344 rats were injected sc with $CdCl_2$ (2.0 mg/kg as Cd) alone or with $CdCl_2$ (2.0 mg/kg as Cd) plus $ZnCl_2$ (10 mg/kg as Zn). The animals were slightly anesthetized with ether and killed by decapitation 24 h after the injection, and then immediately processed as described below.

Absorbance of hemoglobin

To quantify the extent of Cd-induced testicular hemorrhage, 18,000 x g supernatant fraction was used for measurement of the absorbance of hemoglobin at 414 nm by the method of Niewenhuis and Prozialeck (1987) with some slight modifications.

Tissue metal content

The content of Cd in the testes was determined using inductively coupled plasma spectrometry (PerkinElmer Optima 3000 XL) after the tissue was digested with nitric

acid. The contents of Cd in the liver and kidney were determined using atomic absorption spectrometry (Shimadzu AA-6700F) after the digestion. The data was expressed as nanograms or micrograms metal per gram wet weight of tissue.

Determination of testicular metal-binding protein (MT)

Testicular 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-binding protein samples. MT levels were expressed as amount of mercury bound to the protein 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

Cd-induced testicular toxicity

Pathological analysis for the testes of inbred WI and F344 rats were conducted at 24 h after Cd administration. A single injection of Cd at doses of 1.0 and 2.0 mg/kg induced severe hemorrhage in the testes of F344 rats, although the administration of Cd at a dose of 0.5 mg/kg did not cause pathological change (Fig. 1). On the other hand, no significant pathological change was observed in the testes of WI rats treated with the same levels of Cd (Fig. 1).

Figure 2 shows the hemoglobin content of the testicular supernatant, which is a quantitative indicator of Cd-induced hemorrhage in the testes. The hemoglobin content was increased in F344 rats treated with Cd at doses of 1.0 and 2.0 mg/kg, but was not in WI rats, indicating a significant strain difference of the susceptibility to Cd-induced testicular toxicity.

Cd contents in the testes, liver and kidney

In order to elucidate toxicokinetic strain differences in the susceptibility to Cd-induced testicular toxicity, Cd contents in the testes of WI and F344 rats were determined. As shown in Table 1, in the case of the treatment with Cd at a dose of 0.5 or 1.0 mg/kg, the testicular Cd content was not different between the two rat strains. However, when Cd was administered at a dose of 2.0 mg/kg, WI rats exhibited significantly lower Cd content compared to F344 rats, indicating a clear strain difference of Cd accumulation

in the testes. A similar strain difference in Cd accumulation was also observed in the liver and kidney of WI and F344 rats treated with Cd at a dose of 2.0 mg/kg (Table 1).

Effect of Cd treatment on testicular metal-binding protein (MT)

The levels of MT were compared between WI and F344 rats. In all case of control and Cd-treatments, MT levels were significantly higher in WI rats than in F344 rats (Fig. 3). Furthermore, a dose-dependent decrease in testicular MT level was observed in both rat strains.

Effect of co-administered Zn on Cd-induced testicular toxicity

Effect of co-administered Zn on Cd-induced testicular toxicity was examined in F344 rats (Fig. 4). As described above, the single injection of Cd at a dose of 2.0 mg/kg induced severe hemorrhage. However, when Zn (10 mg/kg) was administered in combination with Cd to F344 rats, Cd-induced severe hemorrhage in the testes was reduced. Furthermore, the co-administration of Zn significantly decreased the increased hemoglobin content in the testes of F344 rats treated with Cd (Fig. 5).

Effect of co-administered Zn on Cd contents in the testes, liver and kidney

Since co-administered Zn clearly protected against testicular toxicity of Cd in F344 rats, experiments were performed to determine whether the protective effect is due to altered toxicokinetics of Cd. As shown in Table 2, the testicular Cd content was significantly

decreased by the co-administration of Zn. The hepatic and renal Cd contents were also decreased by the co-administration of Zn (Table 2).

Discussion

The present study examined the strain differences of Cd-induced testicular toxicity in two inbred strains of rats, one which is resistant (WI) and one sensitive (F344) to the acute hepatic toxicity of Cd. The toxic effects of Cd in the testes include severe hemorrhage with edema and hemorrhagic necrosis (Shiraishi and Waalkes, 1996). The results showed that Cd treatment markedly increases testicular necrosis in F344 rats, but does not in WI rats, indicating that WI rats is resistant to Cd-induced testicular toxicity. This strain difference in the testes is consistent with previous reports in which WI rats exhibit a strong resistance to Cd-induced lethality or hepatotoxicity when compared to the more sensitive F344 rats (Shimada et al., 2002, 2004).

Our prior work demonstrated that the hepatic and renal Cd contents are significantly lower after exposure to a toxic dose in WI rats than in F344 rats, suggesting the strong resistance to Cd-induced hepatotoxicity in WI rats is partly associated with a lower hepatic accumulation of the metal (Shimada et al., 2004). Unlike our results, Harstad and Klaassen (2002) reported that although there is a strain differences in Cd-induced hepatotoxicity in male F344 and SD rats, this strain difference cannot be explained on the basis of Cd transport into the liver. Furthermore, King et al. (1998) reported that the accumulation of Cd in the testes of Cd-resistant A/J mice is much lower than that in the testes of Cd-sensitive 129/J mice, although similar difference in Cd accumulation was not observed in the liver and kidney. In the present study, when Cd was

administered at a dose of 2.0 mg/kg, not only in the testes, but also in the liver and kidney, Cd accumulation was confirmed to be significantly lower in WI rats than in F344 rats. It should be noted that there are strain differences in the transport of Cd into the liver and kidney in addition to the testes of WI and F344 rats.

The mechanism by which Cd actually accumulates into the testicular cells is unclear. However, several studies have shown that since Cd and Zn share many physicochemical properties, Cd may be imported into the cells by mimicking Zn at the site of Zn transporters (Waalkes and Poirier, 1985; Waalkes and Perantoni, 1988; King et al., 1999). This is supported by the fact that co-administration of Zn significantly decreases Cd content in the mouse testes while protecting against Cd-induced testicular toxicity (King et al., 1998). In the present study, a similar result was observed in F344 rats. Furthermore, we have shown that the accumulation of Cd by liver slices of WI and F344 rats is decreased by the addition of Zn to the medium (Shimada et al., 2008). Interestingly, in this case, the strain difference of Cd accumulation by liver slices of WI and F344 rats disappeared in the presence of Zn at a higher concentration (Shimada et al., 2008). These results suggest that Zn transport system plays a critical role in strain-related differences of Cd-induced toxicity in WI and F344 rats. Mammalian Zn transporters are classified into two families of ZnT (solute-linked carrier 30; SLC30) and ZIP (ZRT- and IRT-like protein; SLC39) (Palmiter and Huang, 2004; Eide, 2004; Liuzzi and Cousins, 2004). Recently, mouse ZIP8 has been reported as the transporter responsible for strain difference of Cd-induced testicular toxicity in mice (Dalton et al., 2005). Further studies are necessary to identify the transporter(s) that causes strain difference of Cd-induced testicular toxicity in WI and F344 rats.

MT is Cd-binding protein that has been proposed to play an important role in

cellular defense against Cd-induced toxicity (Chellman et al., 1985; Nolan and Shaikh, 1986). In fact, pretreatment with many compounds that are known to stimulate MT synthesis is effective in reducing Cd toxicity (Goering and Klaassen, 1984a; Goering and Klaassen, 1984b). In the present study, testicular MT levels in the control and Cd-treated WI rats were significantly higher than those of the corresponding control and Cd-treated F344 rats. However, where in most tissues MT levels are increased by metal exposure, like Zn or Cd (Goering and Klaassen, 1984a,b), when Cd was administered to WI an F344 rats, testicular MT levels actually decreased in both rat strains in the present work. Consistent with this result, others have shown that testicular MT levels are not increased by the MT inducers, Cd and Zn (Waalkes et al., 1988; Wahba et al., 1994). Furthermore, there is no difference in testicular MT levels between Cd-sensitive and Cd-resistant mouse strains (Nolan and Shaikh, 1986). Thus, accumulating evidence indicates that testicular MT may not in all cases be a critical factor in testicular resistance to Cd. For instance, in rats, progesterone pretreatment markedly increased testicular MT levels while having no effect on Cd-induced testicular toxicity (Shiraishi et al., 1993). Whether or not testicular MT may be involved in this strain difference between WI and F344 rats will require further study.

In conclusion, the results of the present study clearly indicate that there is a clear strain difference in Cd-induced testicular toxicity between inbred WI and F344 rats. This appears to be based on differences in Cd accumulation in the testes. We are currently investigating the molecular mechanism of this difference at the level of the testicular cell.

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Figure legends

Fig. 1 Effect of Cd treatment on pathological changes of the testes in WI and F344 rats. Rats were treated with saline or $CdCl_2$ (0.5, 1.0 or 2.0 mg Cd/kg, sc). Pathological changes were assessed 24 h after the treatment. Typically Cd induced hemorrhagic necrosis in the testes of susceptible rats (F344) but not in resistant rats (WI).

Fig. 2 Effect of Cd treatment on hemoglobin content of the testes in WI and F344 rats as an indicator of hemorrhagic necrosis. Rats were treated with saline or $CdCl_2$ (0.5, 1.0 or 2.0 mg Cd/kg, sc). Hemoglobin content was determined 24 h after the treatment. Data represent the mean ± SEM of 3 or 4 rats. The asterisk indicates a significant difference from WI rats ($P<0.05$) and pound sign indicates a significant difference from the corresponding control ($P<0.05$).

Fig. 3 Effect of Cd treatment on MT levels in the testes of WI and F344 rats. Rats were treated with saline or $CdCl_2$ (0.5, 1.0 or 2.0 mg Cd/kg, sc). MT levels were determined 24 h after the treatment. Data represent the mean ± SEM of 4 or 5 rats. The asterisk indicates a significant difference from WI rats ($P<0.05$) and pound sign indicates a significant difference from the corresponding control ($P<0.05$).

Fig. 4 Effect of co-administered Zn on Cd-induced pathological changes of the testes in F344 rats. Rats were treated with $CdCl_2$ (2.0 mg Cd/kg, sc) alone or with $CdCl_2$ (2.0 mg Cd/kg, sc) plus $ZnCl_2$ (10 mg Zn/kg, sc). Pathological changes were assessed

24 h after the treatment.

Fig. 5 Effect of co-administered Zn on hemoglobin content of the testes in F344 rats as an indicator of hemorrhagic necrosis. Rats were treated with $CdCl_2$ (2.0 mg Cd/kg, sc) alone or with $CdCl_2$ (2.0 mg Cd/kg, sc) plus $ZnCl_2$ (10 mg Zn/kg, sc). Hemoglobin content was determined 24 h after the treatment. Data represent the mean ± SEM of 4 or 5 rats. The asterisk indicates a significant difference from control ($P<0.05$) and pound sign indicates a significant difference from Cd alone ($P<0.05$).