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Strain differences of cadmium-induced hepatotoxicity
in Wistar-Imamichi and Fischer 344 rats: involvement of
cadmium accumulation

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Abstract

We previously reported that Wistar-Imamichi (WI) rats have a strong resistance to cadmium (Cd)-induced lethality compared to other strains such as Fischer 344 (Fischer) rats. The present study was designed to establish biochemical and histological differences in Cd toxicity in WI and Fischer rats, and to clarify the mechanistic basis of these strain differences. A single Cd (4.5 mg/kg, s.c.) treatment caused a significant increase in serum alanine aminotransferase activity, indicative of hepatotoxicity, in Fischer rats, but did not in WI rats. This difference in hepatotoxic response to Cd was supported by pathological analysis. After treatment with Cd at doses of 3.0, 3.5 and 4.5 mg/kg, the hepatic and renal accumulation of Cd was significantly lower in the WI rats than in the Fischer rats, indicating a kinetic mechanism for the observed strain differences in Cd toxicity. Thus, the remarkable resistance to Cd-induced hepatotoxicity in WI rats is associated, at least in part, with a lower tissue accumulation of the metal. Hepatic and renal zinc (Zn) contents after administration were similarly lower in WI than in Fischer rats. When Zn was administered in combination with Cd to Fischer rats, it decreased Cd contents in the liver and kidney, and exhibited a significant protective effect against the toxicity of Cd. We propose the possibility that Zn transporter plays an important role in the strain difference of Cd toxicity in WI and Fischer rats.

Keywords: Cadmium toxicity; Strain difference; Tissue accumulation; Rat
1. Introduction

A nonessential trace element, cadmium (Cd) is an industrial and environmental pollutant. The metal mainly distributes to the liver and kidney in humans and animals (Gregus and Klaassen, 1986; Waalkes et al., 1992; Goering et al., 1994). Acute exposure to Cd produces liver injury, because of the hepatic accumulation of the metal (Dudley et al., 1982). On the other hand, chronic administration of Cd commonly results in renal damage (Kotsonis and Klaassen, 1978; Friberg et al., 1986).

Unlike Cd, metals such as zinc (Zn), iron (Fe), manganese and copper are essential for various biologic processes. However, high levels of these essential metals also induce to toxicity. Thus, cells must maintain tight control of intracellular metal levels. This control is achieved through a balance of efflux and uptake of metals, and is mediated by membrane proteins referred to as metal transporters. Recent studies have shown that mammalian metal transporters play a role in the cellular handling of Cd (Zalups and Ahmad, 2003). For example, divalent metal transporter 1 (DMT1) is involved not only in the cellular uptake of Fe, but also in those of other divalent metals including Cd (Gunshin et al., 1997; Garrick et al., 2003). Zn transporters are also capable of accumulating Cd within mammalian cells (Kumar and Prasad, 2000; Guan et al., 2003). However, the detailed mechanism for cellular uptake of Cd mediated by metal transporters remains to be elucidated.

To examine what transport systems for essential metals can contribute to cellular accumulation of Cd, it is useful to utilize strain differences related to resistance or
susceptibility to Cd toxicity in rodents. Recently, a strain difference in Cd transport to the testis, epididymis and brain of Cd-resistant and -sensitive mice has been elucidated (King et al., 1999), suggesting the involvement of metal transporters in cellular uptake of Cd. Harstad and Klaassen (2002) have also reported that there is a strain difference in sensitivity to Cd-induced hepatotoxicity in male Fischer 344 (Fischer) and Sprague-Dawley (SD) rats, even though this strain difference cannot be explained on the basis of Cd transport to the liver. Our previous paper (Shimada et al., 2002) showed that male Wistar-Imamichi (WI) rats, derived from the Wistar strain, exhibit a strong resistance to the acute toxicity of Cd compared to male Wistar, SD and Fischer rats. Furthermore, we demonstrated that this strong resistance to the acute effects of Cd segregates as an incompletely dominant phenotype in reciprocal crosses between the WI and Fischer strains (Shimada et al., 2003). The purpose of the present study is to establish biochemical and histological differences for Cd toxicity in male WI and Fischer rats, and to clarify factor(s) affecting these strain differences.

2. Materials and methods

2.1. Chemicals

Cadmium chloride (CdCl₂), zinc chloride (ZnCl₂) and iron (II) sulfate heptahydrate (FeSO₄·7H₂O) were purchased from Sigma (St. Louis, MO). All other chemicals were of reagent grade.
2.2. Animals and treatments

Male WI rats were purchased from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan). Male Fischer rats were obtained from Japan SLC (Shizuoka, Japan). 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. CdCl$_2$, ZnCl$_2$ and FeSO$_4$·7H$_2$O dissolved in approximately 0.5 ml of saline were subcutaneously injected into 8-week-old animals. The doses of CdCl$_2$ were 2.0, 3.0, 3.5, 4.5 and 6.5 mg as Cd per kg of body weight. The doses of ZnCl$_2$ were 10 and 20 mg as Zn per kg of body weight, and the dose of FeSO$_4$·7H$_2$O was 20 mg as Fe per kg of body weight. The animals were lightly anesthetized with ether and killed by decapitation 24 h after the administration, and blood and tissues (liver and kidney) were collected. The survival rate (%) was observed for 7 days after administration of Cd (6.5 mg/kg) with or without Zn (10 or 20 mg/kg).

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

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

For histopathological analysis of hepatic damage, the liver was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin. Lesions were assessed by light microscopy.

2.5. Tissue metal content

The contents of Cd, Zn and Fe in the liver and kidney were determined using atomic absorption spectrophotometry with a Shimadzu AA-6400F spectrophotometer after the tissues were digested with nitric acid. The data was expressed as µg metal per g wet weight of tissue.

2.6. Measurement of metallothionein (MT)

The hepatic and renal MT was measured in the animals 24 h after treatment with saline or Cd at a dose of 3.5 mg/kg body weight, according to the method of Naganuma et al. (1987) with a slight modification using non-radioactive HgCl₂ (Yasutake et al., 1998). Briefly, the homogenate was treated successively with diethylmalate and 10 mM CdCl₂, 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 HgCl₂, 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).

2.7. Statistical analysis

Data were analyzed using Student’s $t$-test and $P < 0.05$ was considered to be significant.

3. Results

3.1. Effect of Cd treatment on ALT activity and BUN level

The effects of Cd treatment on serum ALT activity and BUN level, indicators of hepatic and renal damages, were examined in WI and Fischer rats. When Cd was given at a dose of 4.5 mg/kg, the ALT activity was markedly increased in Fischer rats, but was not in WI rats (Fig. 1A), indicating a strain difference in Cd-induced hepatotoxicity. No significant increase in the BUN levels were observed in the two rat strains (Fig. 1B).

3.2. Histology of Cd-induced hepatotoxicity

Figure 2 shows the histological appearance in the liver of rats 24 h after Cd administration. The livers from Cd-untreated WI and Fischer rats were confirmed to be histologically normal (Figs. 2A and 2C). As evident from Fig. 2D, the livers from Fischer rats treated with Cd at a dose of 4.5 mg/kg showed multiple foci of severe
hemorrhagic necrosis. In the livers from WI rats treated with an equal dose of Cd, however, no histological change was observed (Fig. 2B).

3.3. Cd Content after its administration

Strain differences in hepatic and renal Cd contents were examined. As shown in Fig. 3, the hepatic and renal Cd contents were not different between the two strains in Cd-treatment groups at a dose of 2.0 mg/kg. However, when treated with Cd at doses of 3.0, 3.5 and 4.5 mg/kg, WI rats had significantly lower hepatic and renal Cd contents when compared to Fischer rats, indicating a strain difference in Cd accumulation in the liver and kidney.

3.4. Effect of Cd treatment on hepatic and renal MT levels

The hepatic MT levels in WI rats treated with Cd were compared to that in Fischer rats. When Cd was given at a dose of 3.5 mg/kg, the hepatic MT levels were significantly lower in the WI than in the Fischer strain (Fig. 4A). A similar difference was observed between the renal MT levels in WI and Fischer rats treated with Cd (Fig. 4B).

3.5. Zn and Fe contents after their administration

To elucidate the mechanism for strain differences of Cd accumulation in the liver and kidney, Zn was administered to WI and Fischer rats. The hepatic Zn content was significantly lower in WI than in Fischer rats treated with the metal at a dose of 20
mg/kg (Fig. 5A). The renal Zn content was significantly lower in WI than in Fischer rats under all treatments with or without the metal (Fig. 5B). Furthermore, hepatic and renal Fe contents were determined in WI and Fischer rats treated with the metal at a dose of 20 mg/kg (Fig. 6). However, there were no strain differences in the hepatic and renal Fe contents.

3.6. Effect of Co-administered Zn on Cd content and Cd-induced lethality

When Zn (10 or 20 mg/kg) was administered in combination with Cd (3.0 mg/kg) to Fischer rats, the hepatic and renal Cd contents were significantly decreased, as shown in Fig. 7. The co-administered Zn (10 or 20 mg/kg) also induced a significant protective effect against the lethal toxicity of Cd (6.5 mg/kg) in Fischer rats (Fig. 8A). In the case of co-administration of Zn at a dose of 20 mg/kg, all rats of Fischer strain survived (Fig. 8A), similarly to WI rats treated with Cd (6.5 mg/kg) alone (Fig. 8B).

4. Discussion

Several works have shown strain differences of Cd toxicity in rats and mice (Hata et al., 1980; Nolan and Shaikh, 1986; Habeebu et al., 2001; Harstad and Klaassen, 2002). For example, SD rats have been reported to be more resistant than Fischer rats to Cd-induced hepatotoxicity (Harstad and Klaassen, 2002). We have recently shown that WI rats exhibit a strong resistance to acute toxicity of Cd compared to SD rats, even though SD rats are more resistant than Fischer rats to the Cd toxicity (Shimada et al.,
Furthermore, since the WI rat, like Fischer rats, is an inbred rat strain (Sugihara et al., 1996), these strains are useful for analyzing genetically resistance or susceptibility to Cd toxicity. In the present study, we attempted to elucidate the mechanism for this strain difference in Cd toxicity by using the WI and Fischer strains as Cd-resistant and -sensitive rats, respectively.

In the present work measurement of serum ALT activity is the most commonly used procedure for assessment of hepatic damage. The present data showed that Cd treatment markedly increases the enzyme activity in Fischer rats, but does not in WI rats, indicating that the WI strain has a strong resistance to Cd-induced hepatotoxicity. In fact, the strain difference of Cd-induced hepatotoxicity was supported from histological appearance. However, as judged by BUN levels, no significant Cd-induced nephrotoxicity was observed in the two rat strains. This may be because the relative amount of Cd accumulated in the kidney is smaller than that in the liver.

To elucidate whether Cd accumulation in tissues is a basis of the strain differences in the resistance to Cd toxicity, hepatic and renal Cd contents were determined after administration. As expected, the hepatic and renal Cd contents were significantly lower in WI than in Fischer rats. Thus, it appears that the strong resistance to Cd-induced hepatotoxicity in WI rats is associated, at least in part, with a lower accumulation of the metal in the liver. Cd is known to distribute mainly to the liver and kidney in humans and laboratory animals (Gregus and Klaassen, 1986; Waalkes et al., 1992; Goering et al., 1994). However, no published evidence for large strain differences in Cd accumulation in the liver and kidney is available. For instance, in
mice, the hepatic and renal Cd contents are not significantly different in Cd-resistant and -sensitive strains 24 h after Cd administration (King et al., 1998). The present study is the first evidence that strain differences in Cd accumulation in the liver and kidney occur and can contribute to Cd resistance.

Cd induces synthesis of and then binds to MT, a metal-binding protein of low molecular weight (Goering and Klaassen, 1983; Waalkes and Goering, 1990; Klaassen et al., 1999). It was conceivable that the resistance to Cd-induced hepatotoxicity in WI rats was due to a more efficient induction of MT, resulting in higher hepatic MT level in the WI than in the Fischer. However, in this study, the hepatic MT level after Cd administration was significantly lower in the WI rather than in the Fischer strain. Thus, the strain difference of Cd-induced hepatotoxicity does not appear to result from a difference in induction of MT, and MT levels appear to be dictated by accumulated Cd.

Zn and Fe are essential for the growth of various organisms, because they have critical roles in the biochemical and physiological reactions in the cells. The cellular uptakes of Zn and Fe are mediated through specific transport proteins (transporters) to move the metals across the lipid membrane (Gaither and Eide, 2001; Garrick et al., 2003). In the present study, the hepatic and renal Zn contents after administration were significantly lower in WI than in Fischer rats, although there was no strain difference in hepatic and renal Fe contents after administration. The basal Zn content in the liver was similar in untreated WI and Fischer rats (approximately 30 µg/g tissue, see Fig. 5A). Interestingly, when this basal levels was subtracted from those in WI and Fischer rats treated with Zn, the hepatic Zn content in WI rats was found to be about one-half that in
Fischer rats. Furthermore, the co-administration of Zn significantly decreased Cd contents in the liver and kidney of Fischer rats, and caused a significant protective effect against Cd-induced lethality. These results suggest that Zn transporter is likely related to the strain differences in Cd accumulation and toxicity observed in the present study. Because of the similarity in the overall transport characteristics between Zn and Cd, these metals probably share a common transport pathway. A decrease in expression level of Zn transporter or a mutation in primary structure may result in the decreased Cd transport to the liver and kidney in WI rats. In recent studies, a number of Zn transporters have been cloned and characterized in mammals (Palmiter and Findley, 1995; Kirschke and Huang, 2003; Dufner-Beattie et al., 2003; Kambe et al., 2004). We are currently cloning cDNA of Zn transporter responsible for the strain difference in Cd transport to the liver and kidney of WI and Fischer rats.

In conclusion, the present study demonstrates that clear differences in Cd toxicity occur between male WI and Fischer rats, which appears to be based in differences in Cd accumulation in target tissues. This is the first report of strain differences in Cd toxicity in rodents based in Cd toxicokinetics and, as such, provides an important model for further work. It appears that Zn transporters play an important role in this strain difference for Cd toxicity in these rats, although further work is required to confirm this hypothesis.

Acknowledgements
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References


Figure legends

Fig. 1. Effect of Cd treatment on serum ALT activity and BUN level in WI and Fischer rats. Animals were treated with saline or CdCl₂ (4.5 mg/kg as Cd). ALT activity (A) and BUN level (B) were determined 24 h after the treatments. Data represent the mean ± S.D. (n = 3 to 6). **P < 0.01, significantly different from Fischer rats.

Fig. 2. Histological appearance in the liver of WI and Fisher rats 24 h after Cd treatment. Animals were treated with saline or CdCl₂ (4.5 mg/kg as Cd). (A) WI (control); (B) WI (4.5 mg/kg as Cd); (C) Fischer (control); (D) Fischer (4.5 mg/kg as Cd). Note: the severe necrosis of hepatocytes with hemorrhage in (D); no significant changes in (A), (B) and (C); HE x 400.

Fig. 3. Cd contents in the liver and kidney of WI and Fischer rats. Animals were treated with saline or CdCl₂ (2.0, 3.0, 3.5 or 4.5 mg/kg as Cd). Hepatic and renal Cd contents were determined 24 h after the treatments. Data represent the mean ± S.D. (n = 3 to 6). #1, Cd contents in the liver of control WI and Fischer rats were 0.06 ± 0.01 and 0.04 ± 0.01 µg/g tissue, respectively. #2, Cd contents in the kidney of control WI and Fischer rats were 0.05 ± 0.01 and 0.13 ± 0.02 µg/g tissue, respectively. **P < 0.01, significantly different from Fischer rats.
Fig. 4. Effect of Cd treatment on hepatic and renal MT levels in WI and Fischer rats. Animals were treated with saline or CdCl₂ (3.5 mg/kg as Cd). MT levels were determined 24 h after the treatments. Data represent the mean ± S.D. (n = 4 or 6). *P < 0.05, **p < 0.01, significantly different from Fischer rats.

Fig. 5. Zn contents in the liver and kidney of WI and Fisher rats. Animals were treated with saline or ZnCl₂ (10 or 20 mg/kg as Zn). Hepatic and renal Zn contents were determined 24 h after the treatments. Data represent the mean ± S.D. (n = 3 or 4). **P < 0.01, significantly different from Fischer rats.

Fig. 6. Fe contents in the liver and kidney of WI and Fisher rats. Animals were treated with saline or FeSO₄ (20 mg/kg as Fe). Hepatic and renal Fe contents were determined 24 h after the treatments. Data represent the mean ± S.D. (n = 6 to 8).

Fig. 7. Effect of Zn treatment on Cd contents in the liver and kidney of Fischer rats. Animals were treated with CdCl₂ (3.0 mg/kg as Cd) alone or with CdCl₂ (3.0 mg/kg as Cd) plus ZnCl₂ (10 or 20 mg/kg as Zn). Hepatic and renal Cd contents were determined 24 h after the treatments. Data represent the mean ± S.D. (n = 4 to 7). **P < 0.01, significantly different from Fischer rats.

Fig. 8. Effect of Zn treatment on the lethal toxicity of Cd in Fischer rats. Animals were treated with CdCl₂ (6.5 mg/kg as Cd) alone or with CdCl₂ (6.5 mg/kg as Cd) plus
ZnCl₂ (10 or 20 mg/kg as Zn). The toxicity of Cd is expressed as the survival rate (%) 
(n = 5).