A possible mechanism of resistance to cadmium toxicity in male Long-Evans rats

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Abstract

The susceptibility to cadmium (Cd)-induced toxicity in male Long-Evans (LE) rats was compared with that in male Fischer 344 (Fischer) and Wistar-Imamichi (WI) rats, which are sensitive and resistant, respectively, to Cd toxicity. All rats of the LE and WI strains survived for 7 days after the treatment with a toxic dose of Cd (6.5 mg/kg b.w.). However, all rats of the Fischer strain died by the following day. The strong resistance to Cd toxicity in the LE strain was confirmed to be independent of metallothionein synthesis induced by Cd. The hepatic and renal Cd contents after its administration were significantly lower in the LE strain than in the Fischer strain. Furthermore, the hepatic and renal zinc (Zn) contents after its administration were significantly lower in the LE strain than in the Fischer strain. These limited data suggest that the strong resistance to Cd toxicity in male LE rats results from, at least in part, the lower accumulation of the metal in the liver and kidney, in a similar mechanism as the lower Zn accumulation.

Keywords: Cadmium; Resistance; Metallothionein; Long-Evans rat; Tissue accumulation; Zinc

1. Introduction

Although cadmium (Cd) is an industrial and environmental pollutant and is toxic to several tissues such as the liver, kidney and testis, the basis for its toxicity is not yet fully understood. Cadmium initially accumulates in the liver, and therefore acute exposure to toxic doses of Cd produces apotosis and necrosis in the liver (Rikans and Yamano, 2000; Lui and Klassen, 2001).

Our previous paper (Shimada et al., 2002) has demonstrated that male Wistar-Imamichi (WI) rats exhibit a strong resistance to Cd-induced toxicity compared to male Fischer 344 (Fischer) rats. As a mechanism for the strain difference of Cd toxicity, we showed that the accumulation of Cd in the liver is significantly lower in the WI strain than in the Fischer strain (Shimada et al., 2004). The tissue accumulation of Cd can result in oxidative stress and formation of complexes with some biological antioxidants (Rikans and Yamano, 2000; Vido et al., 2001). Tohei et al. (2003) have recently demonstrated that male Long-Evans (LE) and WI rats exhibit high- and low-responses, respectively, to immobilization stress that leads to oxidative injury in various tissues. Interestingly, the high-response to immobilization stress in the LE strain is similar to that in the Fischer strain (Tohei et al., 2003), suggesting that the LE strain is more sensitive as compared to that of the WI strain.

The first aim of the present study was to examine strain differences of Cd toxicity among male LE, Fischer and WI rats. Unexpectedly, the LE strain, unlike the Fischer strain, was strongly resistant to toxic effect of Cd. Thus, we further attempted to

elucidate mechanism for the strong resistance to Cd-induced toxicity in male LE rats.

2. Materials and methods

2.1. Materials

Cadmium chloride ($CdCl_2$) and zinc chloride ($ZnCl_2$) were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of reagent grade.

2.2. Animals and treatment

Male LE and Fischer rats at 8 weeks of age were purchased from KBT Oriental (Saga, Japan) and Japan SLC (Shizuoka, Japan), respectively. Male WI rats at 8 weeks of age were obtained from the Imamichi Institute for Animal Reproduction (Ibaraki, 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. Cadmium chloride or $ZnCl_2$ dissolved in 0.5 ml of saline was subcutaneously injected into each animal. The doses of $CdCl_2$ were 3.5 and 6.5 mg/kg b.w. as Cd, and the dose of $ZnCl_2$ was 20 mg/kg b.w. as Zn. The animals were slightly anesthetized with ether and killed by decapitation 24 h after administration, and tissues (liver and kidney) were excised. The survival rate (%) was observed for 7 days after administration of Cd (6.5 mg/kg b.w.).

2.3. 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 b.w., according to the method of Naganuma et al. (1987) with a slight modification using non-radioactive mercuric chloride ($HgCl_2$) (Yasukake et al., 1998). Briefly, the tissue homogenate was treated diethylmalate and 10 mM $CdCl_2$, then heated at 95˚C for 5 min to precipitate high-molecular weight proteins. After cooling and centrifugation, the supernatant was successively treated with 5 mM $HgCl_2$, 1 mM ovalbumin, and 12.5% trichloroacetic acid. After centrifugation, the supernatant was filtered through a membrane of 0.22 mm pore diameter (Ultrafree C3, Millipore) to afford mercury (Hg)-MT complex samples. MT levels were expressed as amount of Hg bound to thionein molecules after Hg analysis. Mercury levels in the final samples were analyzed by oxygen combustion-gold amalgamation method using an atomic absorption mercury analyzer MD-A (Nippon Instruments Co., Osaka, Japan).

2.4. Measurement of tissue metal content

The contents of Cd and Zn in the liver and kidney were determined using atomic absorption spectrophotometry with a Shimadzu AA-6400F spectrophotometer (Shimadzu Co., Kyoto, Japan) after the tissues were digested with nitric acid.

2.5. Statistical analysis

Data were analyzed using Student's *t*-test and $p < 0.05$ was considered significant.

3. Results

3.1. Susceptibility to Cd toxicity

The susceptibility to Cd toxicity was examined in male LE, Fischer and WI rats. All rats of the LE and WI strains survived for 7 days after the treatment with Cd at a toxic dose of 6.5 mg/kg b.w., as shown in Fig. 1. However, all rats of the Fischer strain died by the following day.

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

The hepatic and renal MT levels in male LE rats treated with Cd at a dose of 3.5 mg/kg b.w. were compared to those in male Fischer and WI rats. There was no significant difference between the hepatic MT levels in the LE and Fischer strains after Cd treatment, even though the hepatic MT level in the WI strain was significantly lower than that in the Fischer strain (Fig. 2A). A similar result was observed among the renal MT levels in the LE, Fischer and WI strains after Cd treatment (Fig. 2B).

3.3. Hepatic and renal Cd contents after its administration

The hepatic and renal Cd contents in male LE rats were compared with those in male Fischer and WI rats. Cadmium at a dose of 3.5 mg/kg b.w. was given to the animals. As shown in Fig. 3A, the hepatic Cd content was significantly lower in the LE and WI strains than in the Fischer strain. In addition, the renal Cd content was significantly

lower in the LE and WI strains than in the Fischer strain (Fig. 3B).

3.4. Hepatic and renal Zn contents after its administration

Figure 4 shows the hepatic and renal Zn contents in male LE, Fischer and WI rats after its administration (20 mg/kg b.w.). The hepatic Zn content was significantly lower in the LE and WI strains than in the Fischer strain (Fig. 4A). A similar result was obtained on a comparison of renal Zn contents (Fig. 4B).

4. Discussion

It has been reported that Cd induces oxidative stress and causes lipid peroxidation by depleting glutathione or by inhibiting antioxidant enzymes such as superoxide dismutase (Hussain et al., 1987; Bagchi et al., 1996; Rikans and Yamano, 2000; Vido et al., 2001). Male LE and Fischer rats have been demonstrated to be sensitive to oxidative stress induced by immobilization, as reported previously (Tohei et al., 2003). However, the LE strain, unlike the Fischer strain, exhibited a strong resistance to Cd-induced toxicity. Based on these observations, it is likely that the strain difference of Cd toxicity cannot be explained by only the susceptibility to oxidative stress.

Cadmium is an effective inducer of MT, which in turn protects host tissues from Cd damage (Goering and Klaassen, 1983; Waalkes and Goering, 1990; Klaassen et al., 1999). Thus, the strong resistance to Cd toxicity in the LE strain may be due to a more efficient induction of MT. However, a significant difference was not observed between

the hepatic MT levels in the LE and Fischer strains after Cd treatment. In addition, the renal MT level in the LE strain was similar to that in Fischer strain. These findings lead us to conclude that the strong resistance to Cd toxicity in the LE strain is independent of MT induction.

In the present study, hepatic and renal Cd contents after its administration were significantly lower in male LE rats than in male Fischer rats. The liver and kidney are important target tissues of Cd (Dudley et al., 1982; Rikans and Yamano, 2000; Lui and Klaassen, 2001). Thus, it is reasonable to assume that strong resistance to Cd toxicity in the LE strain results from the lower accumulation of the metal in the liver and kidney, similar to that in the WI strain (Shimada et al., 2002; Shimada et al., 2004).

Zinc is an essential trace element that is required for a number of biological processes. The intracellular Zn level is strictly regulated by Zn transporters and Zn-binding proteins (Gaither and Eide, 2001; Liuzzi and Cousins, 2004; Kambe et al., 2004). Cadmium and Zn are closely related metals with similar chemical and physical properties and probably share a common transport pathway (Elisma and Jumarie, 2001; Cragg et al., 2001; Zalups and Ahmad, 2003). Our previous paper (Shimada et al., 2004) has shown that hepatic and renal Zn contents after its administration are significantly lower in male WI rats than in male Fischer rats, and further that in male Fischer rats, the hepatic and renal Cd contents after its administration are decreased by co-administration of Zn. In this study, the hepatic and renal Zn contents after its administration were confirmed to be significantly lower in the LE strain than in the Fischer strain. We are currently investigating the expression levels of Zn transporter(s)

responsible for Cd transport to tissue cells of male LE and Fischer rats.

In conclusion, the limited data obtained in the present study provide a possible mechanism for the strong resistance to Cd-induced toxicity in male LE rats.

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Figure legends:

Fig. 1. Susceptibility to Cd toxicity in male LE, Fischer and WI rats. The experiment was performed using 5–6 rats, and Cd at a dose of 6.5 mg/kg b.w. was given to the animals. Cadmium toxicity is expressed as the survival rate (%) in male LE, Fischer and WI rats.

Fig. 2. Effect of Cd treatment on hepatic and renal MT levels in LE, Fischer and WI rats. MT levels were measured 24 h after Cd treatment (3.5 mg/kg b.w.). Data represent the mean ± S.D. of 4–7 rats. *$p < 0.05$; **$p < 0.01$, significantly different from the Fischer strain in the corresponding tissue and dose.

Fig. 3. Hepatic and renal Cd contents in male LE, Fischer and WI rats after its administration. Cadmium content was determined 24 h after its administration (3.5 mg/kg b.w.). Data represent the mean ± S.D. of 5–9 rats. **$p < 0.01$; ***$p < 0.001$, significantly different from the Fischer strain in the corresponding tissue and dose.

Fig. 4. Hepatic and renal Zn contents in male LE, Fischer and WI rats after its administration. Zinc content was determined 24 h after its administration (20 mg/kg b.w.). Data represent the mean ± S.D. of 4–6 rats. **$p < 0.01$, significantly different from the Fischer strain in the corresponding tissue and dose.