Strain difference of cadmium accumulation by liver slices of inbred Wistar-Imamichi and Fischer 344 rats

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Abstract

Strain difference in the accumulation of cadmium (Cd) by liver slices was examined in inbred Cd-resistant Wistar-Imamichi (WI) and Cd-sensitive Fischer 344 (F344) rats. The accumulation of Cd by liver slices of WI rats was significantly lower than that of F344 rats, suggesting strain-related differences in the transport of Cd into the liver cells of these two rat strains. In addition, a similar strain difference was observed in the accumulation of zinc (Zn) by liver slices from WI and F344 rats. Cd accumulation by F344 liver slices decreased when Zn was added to the medium in combination with Cd. Furthermore, in F344 liver slices, Zn accumulation was significantly decreased when Cd was added to the medium. These results suggest that the accumulation of Cd by the liver is probably mediated, at least in part, by Zn transport systems. However, we found no strain difference in hepatic ZnT3 or ZIP3 transcript levels between WI and F344 rats. Further work is in progress to identify the transporter that causes the strain differences in hepatic Cd accumulation seen with WI and F344 rats.

Keywords: Cadmium accumulation; Rat liver slice; Strain difference; Zinc transport system

1. Introduction

Cadmium (Cd) is a highly toxic metal and a common environmental pollutant. In humans and animals, the heavy metal distributes to various tissues such as the liver, kidney and testis, where accumulated Cd can result in tissue damage (Waalkes et al., 1992; Goering et al., 1994). In particular, since Cd accumulates primarily in the liver, acute exposure to toxic doses of Cd produces hepatic necrosis, apoptosis, and eventually death (Rikans and Yamano, 2000; Liu and Klaassen, 2001). Genetic differences in susceptibility to Cd-induced testicular toxicity are known in inbred mouse strains (Taylor et al., 1973; Hata et al., 1980; Chellman et al., 1985; Nolan and Shaikh, 1986). Interestingly, the accumulation of Cd in the testis of Cd-resistant A/J mice was much lower than that in the testis of Cd-sensitive 129/J mice (Nolan and Shaikh, 1986; King et al., 1998, 1999). In the liver or kidney of these two mouse strains, however, no strain difference in Cd accumulation was observed (King et al., 1998). Unlike calcium (Ca) and zinc (Zn), Cd is not essential to living organisms. Thus, transporters of one or more essential metals may be involved in transporting Cd in the testis and other organs.

Harstad and Klaassen (2002) reported strain difference in resistances to Cd-induced hepatotoxicity in Sprague-Dawley (SD) and Fischer 344 (F344) rats. This study (Harstad and Klaassen, 2002) showed that the tissue accumulation of Cd in SD and F344 rats is dependent upon the doses of Cd rather than the strains of rat, indicating that this particular strain difference in Cd-induced hepatotoxicity cannot be explained on the basis of differences of Cd transport. Our recent work demonstrated that the Wistar-Imamichi (WI) strain of rats is strongly resistant to Cd-induced lethality when

compared to F344 rats (Shimada et al., 2002). In this case, however, the hepatic accumulation of Cd was significantly lower in WI rats when compared to F344 rats (Shimada et al., 2004). Furthermore, the co-administration of Zn, which protects against Cd-induced lethality, markedly decreased the accumulation of Cd in the liver of F344 rats (Shimada et al., 2004). These results suggest that Zn transport systems may be the basis of strain-related differences in Cd hepatotoxicity and accumulation in the liver of WI and F344 rats.

Therefore, in the present study, to establish the mechanistic basis of strain difference in Cd-induced hepatotoxicity, we compared the accumulation of Cd by liver slices of inbred WI and F344 rats. Strain difference in the expression of the common hepatic Zn transporter genes ZnT3 and ZIP3 (Liuzzi and Cousins, 2004; Kambe et al., 2004) were also examined in these two rat strains.

2. Materials and methods

2.1. Chemicals

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

2.2. Animals

Inbred male WI (W/Iar) and F344 rats at 8 weeks of age were purchased from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan) and Japan SLC (Shizuoka, Japan), respectively. These animal studies were undertaken in compliance with the guideline principles and procedures of Kumamoto University for the humane care and use of laboratory animals.

2.3. Metal accumulation by liver slices

Metal accumulation experiments were performed as previously described (Yasutake and Hirayama, 2001). WI and F344 rats were slightly anesthetized with ether and euthanized by decapitation. The liver was collected, cut into blocks 1 cm^2 square, and then stored in ice-cold phosphate-buffered saline. Slices about 0.5 mm thick were prepared from each block using a manual tissue slicer (Natsume Co., Tokyo, Japan). All the slices were kept in ice-cold minimum essential medium (pH 7.4) until the start of incubation. The liver slices (n = 3-6 pieces at each point) prepared as above were incubated in the presence or absence of Cd (100 and 250 μM) or Zn (250 and 500 μM) at 37˚C under 95% O_2/5% CO_2 atmosphere for up to 120 min. The concentrations of Cd and Zn in the liver slices were measured by atomic absorption spectrophotometry

with a Shimadzu AA-6400F spectrophotometer (Shimadzu Co., Kyoto, Japan) after digestion with nitric acid.

2.4. Analysis for Zn transporter transcript

$CdCl_2$ was dissolved in saline and injected s.c. (3.0 mg Cd/kg) to rats of each strain (n=3). Four h later, the animals were euthanized as mentioned above, and the liver was excised. Total RNA was extracted from the liver of rats using a commercially available kit (Nippongene, Tokyo, Japan). RNA was quantitated by measuring absorbance at 260 nm and 280 nm. RNA was analyzed to determine the levels of ZnT3 (DDBJ accession No. AY538655; *rattus norvegicus* zinc transporter ZnT-3), ZIP3 (DDBJ accession No. BC086411; *rattus norvegicus* solute carrier family 39 (zinc transporter), member 3) and β-actin (DDBJ accession No. V01217) mRNAs by reverse transcription polymerase chain reaction (RT-PCR).

Primers specific for rat ZnT3, ZIP3 and β-actin cDNAs were purchased from Hokkaido System Science (Sapporo, Japan). The sequences of the forward and reverse primers for ZnT3 are 5′-CTTGAGGGGATGGCCTTC-3′ and 5′-CCTCCGTGGTCTCCCTCT-3′, respectively. The sequences of the forward and reverse primers for ZIP3 are 5′-GCTTTGGTGTGTGACTCTAT-3′ and 5′-GCTGACCTTCCGCCGGGA-3′, respectively. The sequences of the forward and reverse primers for β-actin are 5′-GGCTGTGTTGTCCCTGTAT-3′ and 5′-CCGCTCATTGCCGATAGTG-3′, respectively. The predicted amplified sizes of the ZnT3, ZIP3 and β-actin cDNAs were 322 bp, 398 bp and 351 bp, respectively. PCR was carried out under the following conditions: 95˚C, 10 min for denaturation; 30 cycles (25 cycles for β–actin) at 94˚C for 30 s, 55˚C for 30 s, 72˚C for 1 min for

amplification, then 72˚C for 5 min for extension. After PCR, equivalent amounts of samples were loaded on the 2% agarose gel and separated by electrophoresis.

2.5. Statistical analysis

Statistical analysis was performed using two-sided paired Student's *t*-test, and $p < 0.05$ was considered to be significant.

3. Results

3.1. Strain differences in Cd and Zn accumulation by liver slices

The accumulation of Cd was compared using liver slices of WI and F344 rats. In both strains, the accumulation of Cd by liver slices increased during the incubation in a time-dependent manner (Table 1). Cd accumulation by liver slices of WI rats was generally lower than that of F344 rats, indicating a strain difference of Cd accumulation by rat liver slices. In fact, at the 250 μM level of Cd, the Cd accumulation was significantly less at all times during the incubation (Table 1).

To elucidate a possible mechanism for the strain difference of Cd accumulation, the accumulation of Zn by liver slices of WI and F344 rats was also examined. The accumulation of Zn by liver slices was generally lower in WI rat liver slices, although not significantly so at 250 μM Zn (Table 1). In the presence of Zn at a higher concentration (500 μM), however, the metal accumulation by liver slices of WI rats was significantly lower than that of F344 rats at 30 and 120 min after the incubation (Table 1), providing evidence of a strain difference for Zn accumulation.

3.2. Cd and Zn accumulatiom by liver slices of F344 rats and their mutual effect

Figure 1A shows the effect of Zn on the accumulation of Cd by liver slices of F344 rats. When Zn was added to the medium in combination with Cd, the accumulation of Cd was significantly decreased at all times compared to the accumulation of Cd without Zn added to the medium.

Figure 1B shows the effect of Cd on the accumulation of Zn by liver slices of F344 rats. When Cd was added to the medium, the accumulation of Zn was generally

decreased, and this was significantly so at 60 and 90 min of incubation.

3.3. Effect of Zn on strain-related difference of Cd accumulation by liver slices of WI and F344 rats

Zn (250 or 500 μM) was added to the medium in combination with Cd (250 μM), and the accumulation of Cd by liver slices of WI rats was compared with that of F344 rats. A significant strain-related difference was observed not only in the absence of Zn (Fig. 2A), but also in the presence of Zn (250 μM) (Fig. 2B). However, such a strain-related difference disappeared when Zn at a high concentration (500 μM) was added to the medium (Fig. 2C).

3.4. Hepatic ZnT3 and ZIP3 transcript levels in WI and F344 rats

The levels of ZnT3 and ZIP3 transcripts in the liver of WI and F344 rats were assessed by RT-PCR analysis (Fig. 3). Although the treatment with Cd (3.0 mg/kg) resulted in increased accumulation of both ZnT3 and ZIP3 transcripts, no strain difference in these transcripts was observed between WI and F344 rats.

4. Discussion

We have previously demonstrated that WI rats, compared to F344 rats, exhibit a strong resistance to Cd-induced lethality and hepatotoxicity, and that the accumulation of Cd in the liver of WI rats is significantly lower than that of F344 rats (Shimada *et al*., 2002, 2004). In the present study, further evidence was provided that the accumulation of Cd by liver slices of WI rats is significantly lower than that of F344 rats. These results suggest that there is a strain-related difference in the transport of Cd into the liver cells of WI and F344 rats. On the other hand, Harstad and Klaassen (2002) have shown that the strain differences in resistance to Cd-induced hepatotoxicity in SD and F344 rats cannot be explained on the basis of differences of Cd transport into the liver cells. In order to elucidate the reason for this discrepancy, the strain differences in Cd-induced lethality was re-examined among WI and F344 rats including SD rats (data not shown). All rats of WI strain survived for 7 days after treatment with Cd at a dose of 6.5 mg/kg body weight. In the case of SD strain, however, only 40% of rats tested survived for 7 days after treatment with Cd at the same dose, although all rats of F344 strain died by the following day. Based on these results, it is evident that the resistance to Cd-induced lethality is stronger in WI rats than in SD rats, suggesting the different mechanism for the resistance to Cd-induced hepatotoxicity in these rat strains.

In addition to the strain differences of Cd accumulation described above, the accumulation of Zn by liver slices of WI rats was found to be lower than that of F344 rats at 30 and 120 min of incubation in the presence of the metal at a higher concentration (500 μM). We also demonstrated in this study that the accumulation of Cd by liver slices of F344 rats decreased when Zn was added to the medium in

combination with Cd. Furthermore, Zn accumulation by F344 liver slices decreased when Cd was added to the medium in combination with Zn. Cd and Zn belong to the same column (2B) of the Periodic Table and share some physicochemical properties. Because Cd is a nonessential toxic metal, an endogenous transport mechanism for this metal would not be expected to exist. Thus, it is reasonable to assume that the accumulation of Cd by liver slices of rats is partly mediated by Zn transport system (Zalups and Ahmad, 2003). However, metal transport systems usually have affinities for a variety of metals (Garrick et al., 2003). Further studies are necessary to compare the competitive effects of metals other than Zn on the accumulation of Cd by rat liver slices.

The accumulation of Cd by liver slices of WI rats, like that of F344 rats, decreased when Zn was added to the medium in combination with Cd (see Fig. 2). Interestingly, the strain differences of Cd accumulation by liver slices of WI and F344 rats disappeared in the presence of Zn at a higher concentration (500 μM). These results also suggest that transport mechanism for Zn may play an important role in the strain differences of Cd accumulation by liver slices of WI and F344 rats.

The accumulation of Zn by tissue cells and its transport into and out of intracellular organelles requires transport proteins, *i.e.*, transporters. 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). In an attempt to identify Zn transporter involved in Cd accumulation by rat liver slices, ZnT3 and ZIP3 were chosen as transporters of a preliminary examination. Interestingly, the treatment with Cd resulted in increased levels of hepatic ZnT3 and ZIP3 transcripts in WI and F344 rat strains, although no strain

difference in the expression of hepatic ZnT3 or ZIP3 mRNA was observed. It has been reported that Cd as well as Zn can induce the expression of ZnT1 mRNA in mouse cell lines (Langmade *et al*., 2000). Transporters belonging to ZnT family are known to facilitate the efflux of Zn from cytosol to the outside of cells and transport the cytosolic Zn into intracellular organelles (Liuzzi and Cousins, 2004; Kambe *et al*., 2004). Thus, it is possible that Cd disturbs the homeostasis of Zn in tissue cells, by inducing the expression of ZnT1 and ZnT3 mRNAs. Unlike ZnT-family transporters, ZIP-family transporters function in the influx of Zn into cytosol from the outside of cells and from the lumen of intracellular compartments (Liuzzi and Cousins, 2004; Kambe *et al*., 2004). Recently, mouse ZIP8 has been identified as the transporter responsible for testicular toxicity of Cd (Dalton *et al*., 2005). Furthermore, Fujishiro *et al*. (2006) have shown that the resistance to transport of Cd into metallothionein-null cells may result from the suppressed expression of ZIP14, although whether ZIP14 has the ability to transport Cd remains to be clarified. We are currently identifying the transporter that causes the strain differences in hepatic Cd accumulation seen with WI and F344 rats.

In conclusion, the present study demonstrates that strain differences in the accumulation of Cd by liver slices are observed in inbred WI and F344 rats. In addition, evidence is provided that transport mechanism for Zn is involved, at least in part, in the strain differences of Cd accumulation by liver slices.

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Figure legends

Fig. 1. Cd and Zn accumulation by liver slices of F344 rats and their mutual effect. (A) Liver slices were incubated with 250 μM Cd alone or 250 μM Cd plus 250 μM Zn for up to 120 min. (B) Liver slices were incubated with 250 μM Zn alone or 250 μM Zn plus 250 μM Cd for up to 120 min. Data represent the mean ± SEM (n = 3-6 pieces of liver slices at each point) and the asterisk indicates a significant difference from Cd alone or Zn alone rats ($p < 0.05$).

Fig. 2. Effect of Zn on strain-related difference of Cd accumulation by liver slices of WI and F344 rats. Liver slices were incubated with 250 μM Cd alone (A), 250μM Cd plus 250 μM Zn (B), or 250μM Cd plus 500 μM Zn (C) for up to 120 min. Data represent the mean ± SEM (n = 6 pieces of liver slices at each point) and the asterisk indicates a significant difference from F344 rats ($p < 0.05$).

Fig. 3. Hepatic ZnT3 and ZIP3 transcript levels in WI and F344 rats. Rats were treated with $CdCl_2$ (3.0 mg Cd/kg, s.c.) and the levels were determined 4 h after the treatment. Relative ZnT3 and ZIP3 mRNA expressions were defined as ZnT3 and ZIP3 mRNA accumulations in relation to β–actin mRNA accumulation. Data represent the mean ± SEM (n = 3) and the asterisk indicates a significant difference from control in each rat strain ($p < 0.05$).