Genetic background of resistance to cadmium-induced testicular toxicity in inbred Wistar-Imamichi rats

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Abstract We have previously reported that inbred Wistar-Imamichi (WI) rats are highly resistant to cadmium (Cd)-induced testicular toxicity compared to inbred Fischer 344 (F344) rats. The present study was to elucidate the genetic background of resistance to Cd-induced testicular toxicity in WI rats. The genetic analysis of susceptibility to Cd-induced testicular toxicity was conducted, by using Cd-resistant WI and Cd-sensitive F344 strains as the parental rats, and by using the testicular hemoglobin level as the indicator. In the frequency distribution of testicular hemoglobin levels in parental, first filial (F_1) and second filial (F_2) rats treated with Cd at a dose of 2.0 mg/kg, F_1 rats had testicular hemoglobin levels intermediate to WI and F344 rats, and F_2 rats segregated into three groups of low, intermediate and high phenotypes at the expected ratio. Furthermore, the backcross progeny between WI and F_1 or between F344 and F_1 segregated into two groups with the expected ratio. Based on a simple Mendelian genetic analysis, these segregation patterns lead us to conclude that two co-dominant alleles at a gene locus are responsible for the susceptibility to Cd-induced testicular toxicity in rats. This is the first report for the genetic analysis of susceptibility to Cd-induced testicular toxicity in inbred rat strains.

Keywords Cadmium • Testicular toxicity • Susceptibility • Strain difference • Genetic analysis • Inbred rat

Introduction

The heavy metal cadmium (Cd) is an environmental pollutant that produces toxicity to a variety of mammalian organs such as the liver, kidney and testis (Goering et al. 1994). Among these organs, the testes are extremely sensitive to Cd toxicity. The detailed mechanism(s) for this toxic effect have not yet been elucidated. The onset of Cd toxicity in the testes is rapid, and severe hemorrhage is induced within 24-48 h (Elinder 1986). For example, a marked testicular toxicity, as assessed by pathological change and testicular hemoglobin level, is observed 24 h after the acute administration of Cd (1.0 or 2.0 mg/kg) to Fischer 344 (F344) rats (Shimada et al. 2009).

The toxic effect of Cd in the testes is known to vary with the strain in experimental animals (Gunn et al. 1965; Chellman et al. 1985; King et al. 1999). We have also shown that in Wistar-Imamichi (WI) rats, unlike in F344 rats, the acute administration of Cd (2.0 mg/kg) has little ability to induce testicular toxicity (Shimada et al. 2009). This finding implies that WI rats are highly resistant to Cd-induced testicular toxicity compared to F344 rats. Interestingly, the accumulation of Cd in the testes was significantly lower in WI rats than in F344 rats (Shimada et al. 2009), indicating a toxicokinetic mechanism for the observed strain difference.

So far, evidence has been provided that the susceptibility to Cd-induced testicular toxicity is genetically regulated, by the matings of Cd-resistant C57BL/6J and Cd-sensitive 129/SvJ mouse strains (Taylor et al. 1973; Liu et al. 2001). However, no information on the genetic analysis of susceptibility to Cd-induced testicular toxicity is available in inbred rat strains. In the present study, a simple Mendelian genetic analysis was attempted to elucidate the genetic background of resistance to Cd-induced

testicular toxicity in WI rats.

Materials and methods

Chemicals

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

Animals and treatments

Inbred male and female WI (W/Iar) rats were purchased from Imamichi Institute for Animal Reproduction (Ibaraki, Japan). Inbred male and female F344 (F344/NSlc) rats were purchased from Japan SLC (Shizuoka, Japan). First filial (F_1), second filial (F_2) and backcross generation animals for genetic analysis were obtained from standard cross-matings. The animals were maintained in controlled lighting (12 h light/dark cycle), temperature and humidity, and had free access to a diet of standard laboratory chow and water. $CdCl_2$ (2.0 mg/kg as Cd) dissolved in saline was injected subcutaneously into each animal at 10 weeks of age. The animals were slightly anesthetized with ether and killed by decapitation 24 h after the injection, and the testes were immediately excised. All animal experiments were undertaken in compliance with the guideline principles and procedures of Kumamoto University for the care and use of laboratory animals.

Determination of testicular hemoglobin level

To quantify the extent of Cd-induced testicular hemorrhage, the testicular hemoglobin level was determined by measuring the absorbance of hemoglobin at 414 nm in the 18,000×g supernatant fraction, according to the method of Niewenhuis and Prozialeck (1987) with some slight modifications.

Determination of testicular metal contents

The content of Cd in the testes was determined using inductively coupled plasma spectrometry (Thermo Fisher Scientific ELEMENT) after the tissue was digested with nitric acid. The contents of calcium (Ca) and iron (Fe) in the testes were determined using atomic absorption spectrophotometry with a Shimadzu AA-6700F spectrophotometer after the tissue was digested with nitric acid.

Statistical analysis

For comparison of observed and expected segregation ratios, a classification into low, intermediate and high phenotypes was carried out according to the method of Glowinski and Weber (1982). Inheritance patterns of susceptibility to Cd were analyzed by χ^2 test. Data were analyzed using one-way analysis of variance followed by Tukey's multiple comparison test. The difference at $p < 0.05$ was considered statistically significant. Two-sided test was used in determining statistical significance.

Results

Testicular hemoglobin level and metal contents in parental and F_1 rats treated with Cd

The effects of Cd treatment at a dose of 2.0 mg/kg on testicular hemoglobin level and metal (Ca and Fe) contents were examined in rats of parental (WI and F344) and F_1 generations. The obtained results are summarized in Table 1. The testicular hemoglobin level, which is a quantitative indicator of Cd-induced testicular hemorrhage (Niewenhuis and Prozialeck 1987; Kojima et al. 1992), was significantly lower in WI rats than in F344 rats; the control levels in WI and F344 rats were 0.32 ± 0.01 and 0.32 ± 0.04, respectively. Furthermore, the testicular hemoglobin level in F_1 rats was significantly higher than that in WI rats but significantly lower than that in F344 rats, that is, the testicular hemoglobin level in F_1 hybrids was intermediate to those in the two parental rats. The corresponding toxic effects in the testes of the parental and F_1 rats caused by the treatment with Cd were observed for the contents of Ca and Fe, which are also known as indicators of Cd-induced testicular hemorrhage (Chen and Smith 1992; Kojima et al. 1992). These findings suggest that in rats, the gene defining the susceptibility to Cd-induced testicular toxicity segregates as a co-dominant trait.

Testicular Cd accumulation in parental and F_1 rats

The accumulation of Cd in the testes was determined in parental and F_1 rats treated with its metal at a dose of 2.0 mg/kg. As shown in Fig. 1, WI rats exhibited a significantly low testicular Cd accumulation compared to F344 rats. Furthermore, the testicular Cd

accumulation in F_1 rats was significantly higher than that in WI rats but significantly lower than that in F344 rats, that is, the testicular Cd accumulation in F_1 rats was intermediate to those in the two parental rats.

Frequency distribution of testicular hemoglobin levels in parental, F_1 and F_2 rats treated with Cd

Figure 2 shows the frequency distribution of testicular hemoglobin levels in parental, F_1 and F_2 rats treated with Cd at a dose of 2.0 mg/kg. In the frequency distribution of testicular hemoglobin levels, there was no overlap between parental WI and F344 rats. Rats in F_1 generation exhibited testicular hemoglobin levels of intermediate phenotype which approximate the mean of the two parental phenotypes. Rats in F_2 generation segregated into three groups with a ratio of 11:31:12 (low:intermediate:high) in the frequency distribution of testicular hemoglobin levels. This ratio was not significantly different from the expected ratio of 1:2:1 ($\chi^2 = 1.22$, $p > 0.5$).

Frequency distribution of testicular hemoglobin levels in backcross rats treated with Cd

To establish further the inheritance pattern of testicular hemoglobin levels, we attempted the backcross mating of rats in the parental generation with those in F_1 generation. Figure 3 shows the frequency distribution of testicular hemoglobin levels in the backcross animals treated with Cd at a dose of 2.0 mg/kg. In the frequency distribution of testicular hemoglobin levels, the backcross progeny between F344 and F_1 rats segregated into two groups with a ratio of 10:10 (intermediate:high), which did not

differ significantly from the expected ratio of 1:1 ($\chi^2 = 0$, $p > 1.0$). In addition, although there was an overlap between intermediate and high phenotype, the backcross progeny between WI and F_1 rats appeared to segregate into two groups with a ratio of 6:13 (low:intermediate), which did not differ significantly from the expected ratio of 1:1 ($\chi^2 = 2.58$, $p > 0.1$).

Discussion

It has been reported that in mouse strains, the susceptibility to Cd-induced testicular toxicity cannot be solely explained on the basis of testicular Cd accumulation (Liu et al. 2001). We have previously found that WI rats are highly resistant to Cd-induced testicular toxicity compared to F344 rats (Shimada et al. 2009). This was because the accumulation of Cd in the testes is significantly lower in WI rats than in F344 rats. The present study in rats of parental and F_1 generations has further demonstrated that the level of hemoglobin in the testes is dependent on the accumulation of Cd in the testes, and that the testicular hemoglobin level is a useful means to quantitatively evaluate the extent of Cd-induced testicular hemorrhage (Kojima et al. 1992; Liu et al. 2001). Thus, the genetic analysis of susceptibility to Cd-induced testicular toxicity was conducted, by using inbred Cd-resistant WI and Cd-sensitive F344 strains as the parental rats, and by using the testicular hemoglobin level as the indicator.

As evident from the frequency distribution of testicular hemoglobin levels in parental, F_1 and F_2 rats treated with Cd at a dose of 2.0 mg/kg, F_1 rats had testicular hemoglobin levels intermediate to WI and F344 rats, and F_2 rats segregated into three groups of low, intermediate and high phenotypes at the expected ratio. Based on a

simple Mendelian genetic analysis, these segregation patterns lead us to conclude that two co-dominant alleles at a gene locus are responsible for the susceptibility to Cd-induced testicular toxicity. The results of backcross mating also support this conclusion.

The resistance to Cd-induced testicular toxicity has been considered to be regulated by an autosomal recessive gene, designated *Cdm*, located on chromosome 3 in mice (Taylor et al. 1973), indicating that the Cd-sensitive trait is dominant over the Cd-resistant trait. In fact, Dalton et al. (2000) have pointed out that F_1 generation progenies from the mating of the parental Cd-resistant C57BL/6J and Cd-sensitive DBA/2J mouse strains are, without exception, phenotypically sensitive to Cd-induced testicular toxicity. Recently, the *Cdm* gene has been identified as a member of the solute-carrier superfamily, *Slc39A8* gene (Dalton et al. 2005). The *Slc39A8* gene encodes a Zrt-, Irt-like protein (ZIP)8. ZIP8 is known to function not only as a transport system of zinc (Zn) or manganese, but also as a transport system of Cd responsible for strain difference of Cd-induced testicular toxicity among inbred mouse strains (Wang et al. 2007; He et al. 2009; Siu et al. 2009). As reported previously, we have proposed that a transport system of Zn plays a critical role in strain difference of Cd-induced testicular toxicity in F344 and WI rats (Shimada et al. 2009). This Zn transport system may correspond to ortholog of mouse ZIP8. However, in the present study, the result of genetic analysis demonstrates that the susceptibility to Cd-induced testicular toxicity in rats, unlike that in mice, segregates as a co-dominant trait in F_1 hybrids. Thus, the reason for the differential genetic regulations of susceptibility to Cd-induced testicular toxicity between mice and rats remains to be elucidated.

In conclusion, the segregation patterns of testicular hemoglobin level as the

indicator, based on a simple Mendelian genetic analysis, provide evidence that two co-dominant alleles at a gene locus are responsible for the susceptibility to Cd-induced testicular toxicity in rats. Further studies are in progress to identify the gene defining the resistance to Cd-induced testicular toxicity in WI rats.

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Figure legends

Fig. 1 Accumulation of Cd in the testes of WI, F344 and F_1 rats. Rats were treated with Cd at a dose of 2.0 mg/kg. Data represent the mean ± S.D. of 9 – 12 rats.
* Significantly different from F344 rats ($p < 0.05$). # Significantly different from WI rats ($p < 0.05$).

Fig. 2 Frequency distribution of testicular hemoglobin levels in parental, F_1 and F_2 rats. Rats were treated with Cd at a dose of 2.0 mg/kg. Dotted lines show a classification into low, intermediate and high phenotypes. The classification was carried out according to the method of Glowinski and Weber (1982).

Fig. 3 Frequency distribution of testicular hemoglobin levels in backcross rats. Rats were treated with Cd at a dose of 2.0 mg/kg. Dotted lines show a classification into low, intermediate and high phenotypes. The classification was carried out according to the method of Glowinski and Weber (1982).