D-Amino-acid Oxidase Is Involved in D-Serine-Induced Nephrotoxicity

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Received February 7, 2005

D-Serine is nephrotoxic in rats. Based on circumstantial evidence, it has been suspected that D-amino-acid oxidase is involved in this nephrotoxicity. Since we found that LEA/SENDAI rats lacked D-amino-acid oxidase, we examined whether this enzyme was associated with D-serine-induced nephrotoxicity using the LEA/SENDAI rats and control F344 rats. When D-propargylglycine, which is known to have a nephrotoxic effect through its metabolism by D-amino-acid oxidase, was injected intraperitoneally into the F344 rats, it caused glucosuria and polyuria. However, injection of D-propargylglycine into LEA/SENDAI rats did not cause any glucosuria or polyuria, indicating that D-amino-acid oxidase is definitely not functional in these rats. D-Serine was then injected into the F344 and LEA/SENDAI rats. It caused glucosuria and polyuria in the F344 rats but not in the LEA/SENDAI rats. These results indicate clearly that D-amino-acid oxidase is responsible for the D-serine-induced nephrotoxicity.

Introduction

D-Serine is nephrotoxic in rats. Intraperitoneal injection of D-serine causes necrosis of the cells in the proximal tubules, resulting in glucosuria, aminoaciduria, proteinuria, and polyuria (*1, 2*).

D-Amino-acid oxidase (EC 1.4.3.3) catalyzes the oxidative deamination of D-amino acids, producing the corresponding 2-oxo acids, ammonia, and hydrogen peroxide (*3*). It has a broad substrate specificity and can metabolize many neutral D-amino acids (*4*). This enzyme is present in a wide variety of organisms from yeast to humans (*5*). In vertebrates, it is present in the kidney, liver, and brain; the kidney has the highest concentration in the body. It is located in the peroxisomes of the epithelial cells of the proximal tubules in the kidney (*6, 7*).

D-Amino-acid oxidase is suspected to be involved in D-serine-induced nephrotoxicity because (a) D-serine is reabsorbed in the straight part (pars recta) of the proximal tubules, where cell damage is observed (*1, 2, 8*); (b) D-amino-acid oxidase is localized in this region (*6, 7*); (c) D-serine is a good substrate for this enzyme (*4*); and (d) one of the products of the D-amino-acid oxidase reaction, hydrogen peroxide, is a cytotoxin.

However, there are two perplexing problems. First, D-serine is not nephrotoxic in mice, guinea pigs, rabbits, dogs, hamsters, or gerbils which have levels of D-amino-acid oxidase activity that are comparable to those in rats (*9, 10*). Second, D-alanine, which is also a good substrate for D-amino-acid oxidase, is not nephrotoxic in rats (*10*). These facts cast doubt on the involvement of this enzyme in D-serine-induced nephrotoxicity.

When we were comparing gene expression patterns between Long-Evans cinnamon (LEC) rats and Long-Evans agouti (LEA) rats using microarrays, we found that the expression of the D-amino-acid oxidase gene was extremely low in the LEA rats (*11*). This finding gave us a chance to test the long unresolved problem about whether D-amino-acid oxidase was involved in D-serine-induced nephrotoxicity.

Materials and Methods

Male F344 rats (8 weeks old, 180−210 g) were purchased from Charles River Japan (Yokohama, Japan). LEA/SENDAI rats were maintained at the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine. Male LEA/SENDAI rats (8 weeks old, 208−217 g) were used in these experiments.

Rats were housed in individual metabolic cages 1 day before the injection of D-propargylglycine or D-serine. D-Propargylglycine (Bachem, Bubendorf, Switzerland) was dissolved in 0.85% NaCl at 20 mg/mL and was injected once intraperitoneally into the rats at a dose of 0.2 g/kg. D-Serine (Sigma, St. Louis, MO) was dissolved in 0.85% NaCl at 80 mg/mL and was injected once intraperitoneally into the rats at a dose of 0.8 g/kg. These dosages were chosen according to Nakajima et al. (*12*) and Carone and Ganote (*2*). D-Propargylglycine was injected into four F344 and five LEA/SENDAI rats. D-Serine was injected into five F344 and five LEA/SENDAI rats. Their urine was collected daily

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10.1021/tx0500326 CCC: $30.25
Published on Web 10/21/2005

Table 1. D-Amino-acid Oxidase Activity in the Rat Kidney

strain	activity (nmol/min·mg protein)[a]
F344	6.9 ± 1.2 (5)
LEA/SENDAI	<0.1 (5)

[a] The values represent the mean ± SE. The numbers in parentheses indicate the number of rats used. The enzyme activity of the LEA/SENDAI rats was below the detection limit of our assay.

at 24-h intervals for a total of 8 days. The rats were allowed access to food (Type MF, Oriental Yeast, Tokyo, Japan) and water ad libitum during the experimental periods.

The volume of urine was measured and the glucose content was determined by the method of murotase-glucose oxidase using an assay kit (Wako Pure Chemicals, Osaka, Japan). The significance of the differences in the urinary volume and the glucose content before and after the injection of D-propargylglycine or D-serine was determined using a two-tailed, unpaired *t*-test. A difference of $P < 0.05$ was considered statistically significant.

D-Amino-acid oxidase activity in the kidney homogenates was determined using D-alanine as the substrate as previously described (*13*).

The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Dokkyo University School of Medicine.

Results

D-Amino-acid oxidase activity was determined in the kidney homogenates of LEA/SENDAI rats and F344 rats. Table 1 shows that F344 rats had levels of D-amino-acid oxidase activity comparable to those from other strains of rats (*13, 14*), whereas LEA/SENDAI rats did not have detectable levels of D-amino-acid oxidase activity. These results are in agreement with the results of the microarray experiments, which indicated an extremely low expression of the D-amino-acid oxidase gene in the LEA rats (*11*).

D-Propargylglycine is nephrotoxic in rats and induces glucosuria, aminoaciduria, proteinuria, and polyuria (*12*). It has been shown that metabolism of D-propargylglycine by D-amino-acid oxidase causes necrosis of the cells in the proximal tubules (*15*).

Control male adult F344 rats excreted 5.8 ± 0.9 mL of urine during 24 h (day −1), and their urine contained 0.307 ± 0.074 mg of glucose/mL. Intraperitoneal injection of D-propargylglycine into the F344 rats caused glucosuria (Figure 1A). Urine collected for the first 24 h (day 1) after the D-propargylglycine injection contained 16.7 times more glucose than that collected for the 24 h (day −1) before the injection. The high level of glucose excretion continued and reached its maximum on day 3. The urinary glucose level then declined but was still high even on day 7.

The high level of glucose in the urine was accompanied by an increase in the urinary volume (Figure 2A). The urinary volume after the D-propargylglycine injection increased 2.2 times on day 1. The increase in the urinary volume continued until day 5, and then the urinary volume gradually decreased.

However, when the same amount of D-propargylglycine was injected into male adult LEA/SENDAI rats, glucosuria was not observed (Figure 1B). The urinary volume did not change after the D-propargylglycine injection (Figure 2B), though the basal level of urinary volume was

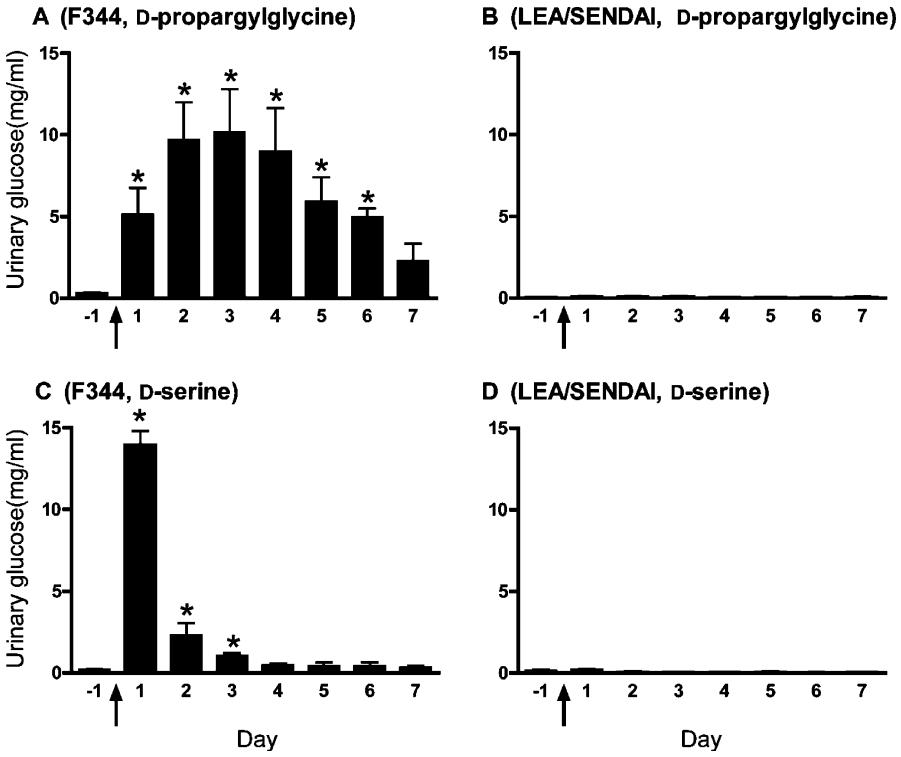

Figure 1. Changes in the glucose content in the urine after D-propargylglycine or D-serine injection. D-Propargylglycine or D-serine was injected intraperitoneally into adult male F344 and LEA/SENDAI rats at 0.2 g/kg or 0.8 g/kg, respectively (arrows). Their urine was collected daily at 24-h intervals for 8 days. The glucose content was determined. The values represent the mean ± SE for four F344 rats and five LEA/SENDAI rats. Asterisks show the significant differences ($P < 0.05$) in the glucose content when the urine was compared with that on day −1. (A) D-Propargylglycine-injected F344 rats. (B) D-Propargylglycine-injected LEA/SENDAI rats. (C) D-Serine-injected F344 rats. (D) D-Serine-injected LEA/SENDAI rats. Note that glucosuria did not occur in LEA/SENDAI rats, which lack D-amino-acid oxidase.

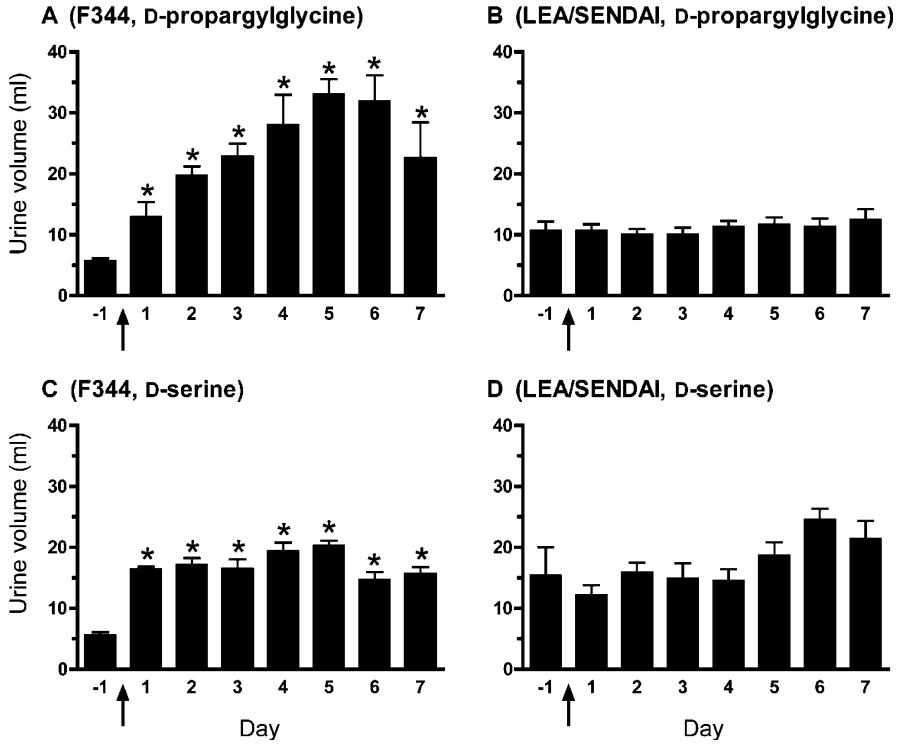

Figure 2. Changes in urinary volume after D-propargylglycine or D-serine injection. D-Propargylglycine or D-serine was injected intraperitoneally into adult male F344 and LEA/SENDAI rats at 0.2 g/kg or 0.8 g/kg, respectively (arrows). Their urine was collected daily at 24-h intervals for 8 days. The volume of urine was measured. The values represent the mean ± SE for five F344 rats and five LEA/SENDAI rats. Asterisks show the significant differences ($P < 0.05$) in the urinary volume when the urine was compared with that on day −1. (A) D-Propargylglycine-injected F344 rats. (B) D-Propargylglycine-injected LEA/SENDAI rats. (C) D-Serine-injected F344 rats. (D) D-Serine-injected LEA/SENDAI rats. Note that polyuria did not occur in LEA/SENDAI rats, which lack D-amino-acid oxidase.

higher in the LEA/SENDAI rats than in the F344 rats (Figures 2A and 2B). These results are similar to those observed in mutant mice lacking D-amino-acid oxidase (*15*). Therefore, D-amino-acid oxidase is not functional in the LEA/SENDAI rats.

D-Serine was then injected into F344 and LEA/SENDAI rats. The injection of D-serine into F344 rats caused glucosuria (Figure 1C). On day 1, the urinary glucose level was 69.3 times higher than that on day −1. However, the urinary glucose level dropped significantly on day 2 and returned to the normal level on day 4. This excretion pattern was different from that observed after the injection of D-propargylglycine (Figure 1A). Polyuria was also observed after the D-serine injection (Figure 2C). The urinary volume on day 1 was 2.9 times larger than that on day −1. The high level of the urinary volume continued until at least day 7.

However, when D-serine was injected into the LEA/SENDAI rats, glucosuria was not observed (Figure 1D) and the urinary volume did not change (Figure 2D).

These results indicate that D-serine-induced nephrotoxicity does not occur in mutant rats that are deficient in D-amino-acid oxidase. Therefore, D-amino-acid oxidase is indispensable for the nephrotoxicity caused by D-serine.

Discussion

It has long been known that D-serine is nephrotoxic in rats (*1, 2*). It damages the straight part of the proximal tubules. Based on circumstantial evidence, it has been proposed that D-amino-acid oxidase is involved in this process. However, no conclusive evidence has, so far, been obtained. The present experiments showed that D-serine provoked neither glucosuria nor polyuria in the mutant rats that lack D-amino-acid oxidase activity, indicating that this enzyme is involved in D-serine-induced nephrotoxicity.

It is known that D-propargylglycine is nephrotoxic in rats and mice (*12, 15*). It has been shown that metabolism of D-propargylglycine by D-amino-acid oxidase is the cause of this nephrotoxicity (*15*). Since the same phenomenon was observed in the D-serine-induced nephrotoxicity, it is very likely that the metabolism of D-serine by D-amino-acid oxidase is responsible for this kidney damage. These results strongly suggest that the common metabolite produced in the metabolisms of D-propargylglycine and D-serine is the real nephrotoxic substance. Hydrogen peroxide and ammonia are the common products in these reactions. Since ammonia is not a significant nephrotoxic substance unless its concentration is very high, hydrogen peroxide and the concomitantly produced oxygen radicals are likely to be the toxic substances. The fact that co-injection of D-serine and reduced glutathione, which captures oxygen radicals, prevented D-serine-induced aminoaciduria (*16*) supports this hypothesis.

However, the causal role of hydrogen peroxide has to be carefully considered. Although D-alanine is a good substrate for D-amino-acid oxidase and produces hydrogen peroxide, it is not nephrotoxic in rats (*10*). This discrepancy has to be resolved. Recently, Park et al. (*17*) have found that D-serine was cytotoxic to rat glial cells in culture. They found that D-amino-acid oxidase was involved in this process since simultaneous addition of inhibitors against D-amino-acid oxidase attenuated the cytotoxic effect of D-serine and, on the contrary, addition of an inhibitor to catalase, which decomposes hydrogen

peroxide, enhanced the cytotoxic effect. They concluded that hydrogen peroxide was a causative agent. The cytotoxicity was specific to D-serine and was not observed in D-alanine or D-proline. They ascribed these differences to the transport system for each D-amino acid.

Another problem is that D-serine-induced nephrotoxicity occurs only in rats. Guinea pigs, rabbits, mice, dogs, hamsters, and gerbils have D-amino-acid oxidase and, therefore, hydrogen peroxide should also be produced in their kidneys when D-serine is injected. However, they are entirely free from the nephrotoxicity (*9, 10*). The level of D-amino-acid oxidase activity does not appear to be the critical factor since dogs have a higher activity than rats, whereas guinea pigs and mice have lower activities than rats (*10, 13*). Reabsorption and transport of D-serine may be different between rats and other animals. Indeed, Huang et al. (*18*) found that urinary excretion of D-serine was significantly lower in rats than humans and dogs, though plasma D-serine concentrations were not so different among these animals. In addition, it is known that rats have a higher capacity to utilize D-amino acids than other animals (*5*). Utilization of D-amino acids is initiated by D-amino-acid oxidase (*19, 20*). Conversion of D-amino acids to 2-oxo acids mainly takes place in the kidney (*20*). Therefore, these facts may explain why rats are more susceptible to D-serine-induced nephrotoxicity than other animals.

Injections of D-propargylglycine and D-serine caused different excretion patterns of glucose in F344 rats (Figures 1A and 1C). The increase in the urinary volume was also different after D-propargylglycine and D-serine injections (Figures 2A and 2C). Since the same cells in the proximal tubules are believed to be damaged by these substances via metabolism by D-amino-acid oxidase, similar changes in urinary glucose and volume would be expected to occur. However, it seemed that D-propargylglycine caused a more severe nephrotoxicity than D-serine. Since only a single dose was used for each substance, it is not clear at present whether this merely reflects a difference in the concentration of the nephrotoxic substances.

After the D-serine injection into the F344 rats, the glucose content in the urine was very high on day 1 and then rapidly decreased on day 2 and day 3 (Figure 1C). However, the urinary volume remained high until day 7 (Figure 2C). The same pattern was observed in D-serine-injected Sprague-Dawley rats (*2*). It is likely that the recovery rate from the damage to the reabsorption mechanisms for glucose and water is different.

The F344 rats were used as a control for the LEA/SENDAI rats in the present experiments. However, since these strains probably have many genetic differences besides D-amino-acid oxidase levels, a genetically closer strain of rats should have been used as a control. LEC rats are genetically closer to the LEA rats but they could not be used because they have a serious liver disease (*11*). The use of the F_2 segregants from a genetic cross between the F344 and LEA/SENDAI rats is the best choice for these experiments. However, D-serine-induced nephrotoxicity occurred not only in F344 rats (*21,* present experiments) but also in Sprague-Dawley rats (*1, 2, 10*), Wistar rats (*16*), and Alderley Park rats (*22*), whereas it was not observed in the LEA/SENDAI rats. The only obvious difference among these strains of rats is the presence or absence of D-amino-acid oxidase, suggesting that this enzyme is responsible for the nephrotoxicity. Williams and Lock (*23*) recently found that pretreatment of Alderley Park rats with sodium benzoate, a competitive inhibitor of D-amino-acid oxidase, resulted in a dose-dependent reduction of D-serine-induced nephrotoxicity. These observations seem to support our contention.

D-Serine is present in human blood (*24, 25*) and urine (*26*). Interestingly, it is present at a high concentration in the brain of humans (*27−29*). It is not known whether humans suffer from D-serine-induced nephrotoxicity like rats or are free from it like guinea pigs, rabbits, mice, dogs, hamsters, and gerbils. Until this is clearly determined, it might be prudent to consider D-serine as a potentially hazardous substance for humans.

Acknowledgment. This work was supported in part by a grant from the Asahi Glass Foundation.

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TX0500326