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Inhibition of γ -Glutamyl Transpeptidase or Cysteine *S*-Conjugate β -Lyase Activity Blocks the Nephrotoxicity of Cisplatin in Mice

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Abstract

Cisplatin is nephrotoxic. The mechanism underlying this organ-specific toxicity is unknown. We hypothesize that cisplatin is metabolized via a γ -glutamyl transpeptidase (GGT) and cysteine *S*-conjugate β -lyase-dependent pathway that has been shown to activate several haloalkenes to nephrotoxins. To test this hypothesis, we inhibited GGT and cysteine *S*-conjugate β -lyase in C57BL/6 mice and analyzed the effect of the inhibitors on the nephrotoxicity of cisplatin. GGT was inhibited by pretreating the mice with acivicin. Cysteine *S*-conjugate β -lyase was inhibited by aminooxyacetic acid (AOAA). Male C57BL/6 mice were treated with 15 mg/kg cisplatin (i.p.) and sacrificed on day 5. Half the mice treated with cisplatin alone died before sacrifice. The cisplatin-treated mice sacrificed at 5 days had significantly elevated levels of blood urea nitrogen (BUN). Histologic analysis revealed severe damage to the renal proximal tubules. Pretreatment with acivicin or AOAA protected the mice from the nephrotoxicity of cisplatin. None of the pre-treated animals died before sacrifice. BUN levels and quantitative histologic analysis of the kidneys confirmed the protective effect of acivicin and AOAA. Platinum levels in the kidneys were not altered by acivicin or AOAA, indicating that neither affected the uptake of cisplatin into the kidney. Likewise, cisplatin-induced weight loss was not altered by acivicin or AOAA, suggesting that weight loss and nephrotoxicity are via distinct mechanisms. These data support the hypothesis that the nephrotoxicity of cisplatin is due to the metabolism of a platinum-glutathione conjugate by GGT and cysteine *S*-conjugate β -lyase to a potent nephrotoxin.

Cisplatin is a widely used chemotherapy drug. Nephrotoxicity is one of its dose-limiting side effects (Madias and Harrington, 1978). Our previous studies showed that in rats, inhibition of γ -glutamyl transpeptidase (GGT) activity blocked cisplatin-induced nephrotoxicity (Hanigan et al., 1994). Inhibition of GGT also inhibits the nephrotoxicity of halogenated alkenes and quinones such as trichloroethene, hexachloro-1,3-butadiene, 2-bromo-2-chloro-1,1-difluoroethene, and 2-bromohydroquinone (Lau and Monks, 1990; Finkelstein et al., 1992; Lash et al., 2001). These compounds form glutathione *S*-conjugates that are metabolized by GGT in the kidney to nephrotoxins (Anders and Dekant, 1998; Lash et al., 2001). The renal toxicity of both classes of compounds is localized to the proximal tubule cells, the same cells killed by cisplatin (Dekant, 1993; Hanigan et al., 1994). Although the

metabolic activation of both the halogenated alkenes and the hydroquinones is initiated by the formation of a glutathione-conjugate and the extracellular cleavage by GGT, the further metabolism of these two classes of compounds diverges downstream of the GGT reaction. The nephrotoxicity of the halogenated alkenes can be inhibited by aminooxyacetic acid (AOAA), which inhibits cysteine *S*-conjugate β -lyase activity (Elfarra et al., 1986; Finkelstein et al., 1992), whereas AOAA has little effect on the nephrotoxicity of the hydroquinones (Dekant and Vamvakas, 1996). In this study we sought to determine which, if either, of these pathways activates cisplatin. We tested the hypothesis that cisplatin is metabolized to a nephrotoxin via the GGT-dependent and cysteine *S*-conjugate β -lyase-dependent pathway that activates the halogenated alkenes to nephrotoxins.

The structures of trichloroethene and hexachloro-1,3-butadiene are shown in Fig. 1A. Trichloroethene is metabolized by several pathways. Its conversion to a nephrotoxin is initiated in the liver, where it is conjugated to glutathione, yielding *S*-(1,2-dichlorovinyl)glutathione (Lash et al., 2000). Hexachlorobutadiene is conjugated to glutathione yielding 1-(glutathio-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (Koob and Dekant, 1992). The glutathione *S*-conjugates of the drugs are further metabolized to nephrotoxins in the kidney (Anders and Dekant, 1998; Dekant and Henschler, 1999). The glutathione *S*-conjugates are cleaved by GGT, which is expressed on the surface of the proximal tubule cells. GGT hydrolyzes the glutathione *S*-conjugates to cysteinyl-glycineconjugates. Amino-dipeptidase, another cell surface enzyme, hydrolyzes the cysteinyl-glycine-conjugates to cysteine-conjugates. The resulting cysteine-conjugates are taken up into the cell where they can be metabolized by cysteine *S*-conjugate β -lyase, resulting in the formation of an unstable thiol, which is a highly reactive compound. The cysteine-conjugates can also be converted by *N*-acetyl transferase to mercapturic acids, which are not toxic and are excreted in the urine. The localized toxicity of the nephrotoxic halogenated alkenes can be explained by the cell specific expression of the enzymes involved in the metabolism. In the kidney, GGT and cysteine *S*-conjugate β -lyase activities have been localized to the proximal tubules (Hanigan and Frierson, 1996; Kim et al., 1997).

The anti-tumor activity of cisplatin is attributed to the formation of platinum-DNA adducts (Trimmer and Essig-mann, 1999). When cisplatin enters the cell, the chloride ions become displaced due to the low intracellular chloride concentration. The resulting positively charged platinum ion binds DNA and other negatively charged groups. The platinum forms intra- and interstrand DNA crosslinks. In dividing cells, the DNA crosslinks are toxic because they inhibit DNA replication. The proximal tubule cells, the target of cisplatin-induced renal toxicity, do not divide in an adult. Therefore, the formation of DNA adducts cannot account for the nephrotoxicity of the drug. Data from several laboratories are consistent with the hypothesis that cisplatin is metabolized to a nephrotoxin through a glutathione-conjugate. Daley-Yates and McBrien identified several platinum-containing species in the plasma of rats after cisplatin treatment that were more nephrotoxic than cisplatin (Daley-Yates and McBrien, 1984). Pretreating rats with ketoprofen, an inhibitor of glutathione *S*-transferase, significantly reduced the nephrotoxicity of cisplatin (Sadzuka et al., 1994). The kidneys of cisplatin-treated rats have been shown to contain cysteine-platinum conjugates (Mistry et al., 1989).

Our hypothesis is that cisplatin is conjugated to glutathione and metabolized to a nephrotoxin via the same pathway that activates the nephrotoxic, halogenated alkenes. The proposed metabolism of cisplatin is shown in Fig. 1B. Investigators studying the metabolism of the halogenated alkenes identified the route by which these drugs were activated by inhibiting GGT and cysteine *S*-conjugate β -lyase in vivo before administration of the drugs. Acivicin was used as an inhibitor of GGT and AOAA was used to inhibit cysteine *S*-conjugate β -lyase (Elfarra et al., 1986; deCeaurreiz and Ban, 1990; Lash et al., 1994). To test our hypothesis, we treated mice with these same inhibitors before cisplatin treatment and assessed the effect of the inhibitors on cisplatin-induced renal damage.

Materials and Methods

Animals.

Male C57BL/6 mice (6–8 weeks old) were purchased from Harlan (Indianapolis, IN). Animals were housed in hanging cages in the Animal Resource Facilities at the University of Virginia. Food and water were provided ad libitum.

Drugs and Chemicals.

Acivicin (Sigma Chemical, St. Louis, MO) was dissolved in saline (0.9% sodium chloride) at 5 mg/ml. AOAA (Sigma) was dissolved in saline at 10 mg/ml. The solutions were prepared and sterilized by filtration through a 0.22- μ m filter within 30 min of treatment. Cisplatin was purchased as a sterile solution, 1 mg/ml saline (Bristol-Myers Laboratories, Evansville, IN).

Inhibition of GGT Activity by Acivicin.

GGT activity was inhibited by acivicin as previously described (Ban et al., 1994). Briefly, acivicin was dissolved in saline at 5 mg/ml. Mice were treated with 50 mg of acivicin/kg body weight at 1 h (oral gavage) and 30 min (i.p.) before cisplatin treatment. Immediately before cisplatin treatment, one mouse from the saline treatment group and one mouse from the acivicin treatment group were sacrificed to assess the GGT activity in the kidney. The kidneys were removed, frozen on dry ice, and stored at -80°C . To determine GGT activity, the kidneys were thawed and homogenized with a Potter-Elvehjem homogenizer in 0.1 M bicine buffer (pH 8.6). The homogenates were assayed for GGT activity as previously described (Hanigan and Frierson, 1996). One unit of GGT activity was defined as the amount of enzyme that released 1 μ mol of *p*-nitroaniline per min at 25°C . The protein concentration in the homogenate was determined with the BCA protein assay (Pierce, Rockford, IL).

Inhibition of Cysteine *S*-Conjugate β -Lyase Activity by AOAA.

AOAA is an inhibitor of pyridoxal 5'-phosphate-containing enzymes (Cooper, 1994). It has been shown to inhibit cysteine *S*-conjugate β -lyase activity both in vivo and in vitro (Elfarra et al., 1986). The AOAA protocol described by de Ceaurreiz and Ban (1990) for inhibiting cysteine *S*-conjugate β -lyase activity in mice was used. Briefly, AOAA (100 mg/kg b.wt.) was administered by oral gavage 1 h before, 10 min before, and 5 h after cisplatin treatment.

Experimental Protocol.

The mice were divided into six groups of 8 to 11 mice per group. To control for the stress of handling and for the volume of saline injected in the mice, the mice that were not treated with acivicin or AOAA were administered equivalent doses of saline (10 μ l/g b.wt.). The mice that were not treated with cisplatin were treated with an equivalent dose of saline (15 μ l/g b.wt.). The mice were treated as follows: group 1, the saline treatment group ($n = 8$) was administered three doses of saline (10 μ l/g b.wt.) via oral gavage, 1 h before, 10 min before and 5 h after 15 μ l of saline/g b.wt.(i.p.). Group 2, the acivicin treatment group ($n = 8$) was administered 50 mg of acivicin/kg b.wt. 1 h (oral gavage) and 30 min (i.p.) before 15 μ l of saline/g b.wt. (i.p.). Group 3, the AOAA treatment group ($n = 8$) was administered three doses (oral gavage) of 100 mg of AOAA/kg b.wt. treatments 1 h before, 10 min before, and 5 h after 15 μ l of saline/g b.wt. (i.p.). Group 4, the cisplatin treatment group ($n = 11$) was administered three doses of saline (10 μ l/g b.wt.) via oral gavage 1 h before, 10 min before, and 5 h after 15 mg of cisplatin/kg b.wt.(i.p.). Group 5, the acivicin cisplatin treatment group ($n = 8$) was administered two doses of 50 mg of acivicin/kg b.wt. given 1 h (oral gavage) and 30 min (i.p.) before treatment with cisplatin (15 mg/kgb.wt., i.p.). Group 6, the AOAA cisplatin treatment group ($n = 8$) was administered three doses of 100 mg of AOAA/kg b.wt. (oral gavage) 1 h before, 10 min before, and 5 h after treatment with cisplatin (15 mg/kg b.wt., i.p.).

Five days after treatment, the mice were weighed then sacrificed by decapitation and blood was collected into a 15-ml tube. Serum was prepared for blood urea nitrogen (BUN) analysis. Kidneys were removed and weighed. The left kidney was stored (-80°C) and used for platinum analysis, and the right kidney was fixed for histologic analysis.

BUN Assays.

BUN levels were measured in serum with the colorimetric, end-point BUN diagnostic K #16–11(Sigma).

Histology.

Kidneys were fixed in Bouin's fixative, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin (Davenport, 1960). Kidney damage was quantitated by recording the number of proximal tubules that contained protein casts in each 10 $\times$ field. A minimum of ten 10 $\times$ fields were analyzed per kidney section.

Platinum Analysis.

Platinum levels were quantitated with a SPECTRAA-220Z graphite furnace double beam atomic absorption spectrophotometer with Zeeman background correction (Varian, Houston, TX). Kidney tissue was digested at 70°C in concentrated nitric acid (250 mg tissue/ml nitric acid). Samples were diluted 1:3 with distilled water. The platinum standard included equivalent amounts of nitric acid.

Data Analysis.

The means and standard deviation (S.D.) from the mean was computed for each treatment group. A one way analysis of variance was used to determine whether there were significant differences between the treatment groups. Dunnett's test was used to compare each group with the saline-treated control. Tukey's test was used to make all pairwise comparisons. Significant differences in mortality between groups were detected by a pairwise comparison of groups with the Fisher's exact test.

Results

Inhibition of Renal GGT Activity with Acivicin.

To confirm the inhibition of GGT activity by acivicin, mice from the saline treatment group and the acivicin treatment group were sacrificed immediately before cisplatin treatment. Kidneys from the saline treatment group had 528 ± 108 milli-units of GGT activity/mg of protein. Kidneys from the acivicin treatment group had 29 milliunits of GGT activity/mg of protein. Acivicin decreased renal GGT activity by 96% in agreement with previous studies (deCeaurrez and Ban, 1990).

Effect of Acivicin and AOAA on Survival of Cisplatin-Treated Mice.

Six of the 11 mice in the cisplatin treatment group died before day 5 (Table 1). The mice began dying on day 3. Pretreatment with acivicin or AOAA protected against cisplatin-induced toxicity. There were no deaths among the eight mice in the acivicin cisplatin treatment group or seven mice in the AOAA cisplatin treatment group, demonstrating a significant protective effect of each of these two inhibitors ($p < 0.04$). None of the mice in any of the three control groups, saline, acivicin, or AOAA treatment groups died before sacrifice on day 5. Two mice sustained lung punctures during oral gavage of AOAA and died the same day as the treatment. One mouse was in the AOAA treatment group and one was in the AOAA cisplatin treatment group. They were excluded from the analysis.

Effect of Treatment on Body Weight.

Pretreatment with acivicin or AOAA before cisplatin treatment did not affect cisplatin-induced weight loss among those animals that survived 5 days after treatment with cisplatin. Each animal was weighed before initial treatment and again before sacrifice at day 5. The percentage change in body weight was calculated for each animal, and the average for each group is shown in Table 2. Treatment with cisplatin caused a 24.3% reduction in body weight, a significant change relative to saline-treated controls ($p < 0.05$). Neither acivicin nor AOAA protected against the cisplatin-induced weight loss. The mice in these groups lost $20.4\% \pm 3$ and $17.1\% \pm 3$ of their body weight. Acivicin or AOAA treatment alone did not significantly effect body weight relative to saline-treated controls. The average percent weight change among mice in the acivicin and AOAA treatment groups was $-0.9\% \pm 1$ and $\pm 3.6\% \pm 1$, respectively.

Serum BUN Levels 5 Days after Treatment.

Serum was collected at the time of sacrifice and BUN levels were measured to assess renal damage (Fig. 2). The mice that survived 5 days in the cisplatin treatment group had the highest BUN values, 32.8 ± 1 mg/dl, a significant elevation relative to all other treatment groups ($p < 0.05$). Acivicin was protective against cisplatin-induced nephrotoxicity. The acivicin cisplatin treatment group had an average BUN value of 18.3 ± 9 mg/dl. This value was significantly lower than the cisplatin treatment group ($p < 0.05$), although it was significantly higher than the saline treatment group indicating that acivicin did not provide complete protection ($p < 0.05$). This low level of toxicity may be due to the low level of GGT activity still present in the kidney, 29 milli-units/mg of protein (4% of normal levels) in the acivicin-treated mice. AOAA completely blocked cisplatin-induced nephrotoxicity. The AOAA cisplatin treatment group had an average BUN value of 5.9 ± 4 mg/dl, which did not differ from the saline treatment group. Treatment with acivicin or AOAA alone did not effect BUN. The BUN value for the acivicin treatment group and the AOAA treatment group were not significantly different from the saline treatment group, 6.7 ± 2.6 , 3.5 ± 1.7 , and 8.3 ± 2.5 mg/dl, respectively.

Renal Histology.

The nephrotoxicity of cisplatin and the protective effects of acivicin and AOAA against cisplatin-induced damage were confirmed by qualitative and quantitative histologic analysis of the kidneys excised 5 days after treatment. Qualitative histologic analysis of the kidneys in the saline treatment group showed normal proximal tubules having prominent brush borders and clear lumen (Fig. 3A). The morphology of the acivicin treatment group and the AOAA treatment group were indistinguishable from the saline treatment group. Analysis of the kidneys from mice in the cisplatin treatment group revealed extensive tubular necrosis, loss of the epithelial brush-border, and the presence of protein casts in the lumen (Fig. 3B). Kidneys from mice in the acivicin cisplatin treatment group showed minor renal damage as measured by minor tubular changes in morphology, intact epithelial brush border, and the presence of fewer protein casts (Fig. 3C). Kidneys from mice in the AOAA cisplatin treatment group had normal morphology and were indistinguishable from the saline treatment group (Fig. 3D).

To quantitatively confirm the histologic damage, protein casts, which are indicators of tubular damage, were counted in each kidney (Table 3). No protein casts were observed in the kidneys of mice from the saline treatment group, acivicin treatment group or the AOAA treatment group. Kidneys of the mice from the cisplatin treatment group had significantly more protein casts than the saline treatment group (9.8 ± 1.1 versus 0 ± 0 protein casts/10 $\times$ field, $p < 0.05$). Kidneys of the mice from the acivicin cisplatin treatment group had significantly fewer protein casts than the cisplatin treatment group (1.2 ± 1.1 versus 9.8 ± 1.1 protein casts/10 $\times$ field, $p < 0.05$); however, the acivicin cisplatin treatment group did have significantly more protein casts than the saline treatment group. This lack of complete protection was again likely the result of the low level of GGT activity in the acivicin-treated groups. The AOAA cisplatin treatment group was indistinguishable from the saline treatment group with 0 ± 0 protein casts/10 $\times$ field. These data were consistent with the degree of renal damage in each treatment group as assessed by serum BUN levels.

Analysis of Platinum Levels in the Kidneys.

The protective effect of acivicin and AOAA was not due to a reduced accumulation of platinum in the kidneys of mice treated with cisplatin. Platinum levels in the kidneys of mice sacrificed 5 days after treatment were determined by graphite furnace atomic absorption spectroscopy (Table 4). No significant differences in platinum accumulation were observed between the cisplatin treatment group, acivicin cisplatin treatment group, and AOAA cisplatin treatment group, 8.0 ± 1.8 , 8.6 ± 1.5 , and $7.0 \pm 0.4 \mu\text{g}$ of platinum/mg of kidney, respectively. Platinum was not detected in the kidneys of mice in the saline, acivicin, or AOAA treatment groups (data not shown).

Discussion

In this study we investigated the mechanism by which cisplatin is toxic to renal tubule cells. BUN values and histologic analysis showed that pretreating mice with acivicin or AOAA significantly reduced the nephrotoxic effects of cisplatin. These findings supported our hypothesis that cisplatin is metabolized in the kidney by a GGT and cysteine *S*-conjugate β -lyase-dependent pathway. Renal accumulation of platinum was not altered by acivicin or AOAA, indicating that these compounds are inhibiting nephrotoxicity without blocking the uptake of cisplatin into the kidney.

The inhibition of cisplatin nephrotoxicity by AOAA supports our hypothesis that the metabolism of cisplatin is via the same pathway as the halogenated alkenes. This pathway differs from that of the hydroquinones, which are nephrotoxic in the presence of AOAA (Monks et al., 1988; Fowler et al., 1994). AOAA is a pyridoxal 5'-phosphate (PLP) antagonist. There are several other families of PLP-dependent enzymes in addition to the PLP-dependent cysteine *S*-conjugate β -lyases (Mehta and Christen, 2000). We cannot exclude the possibility that PLP-dependent enzymes other than cysteine *S*-conjugate β -lyase were inhibited by AOAA and are necessary for the nephrotoxicity of cisplatin. However, we have shown that AOAA, at the same dose that inhibits the nephrotoxicity of halogenated alkenes, also inhibits the nephrotoxicity of cisplatin. There is evidence, independent of AOAA, that cysteine *S*-conjugate β -lyases catalyze the activation of the halogenated alkenes (Abraham et al., 1995; Kim et al., 1997).

The proteins that catalyze the cysteine *S*-conjugate β -lyase reaction differ among organs. In rat liver, the cysteine *S*-conjugate β -lyase enzyme has been shown to be identical with kynureninase (Stevens, 1985). In rat kidney, two enzymes having cysteine *S*-conjugate β -lyase activity have been identified. One is a 90-kD dimer that has been shown to be identical with cytosolic glutamine transaminase K (Stevens et al., 1986). The second has an apparent molecular mass of 330 kD and is localized to the mitochondria (Abraham et al., 1995). Abraham and coworkers (1995) have presented evidence that the cysteine-conjugates of the halogenated alkenes are substrates of the high-molecular weight enzyme. Immunohistochemical experiments have demonstrated that the electrophilic metabolites of the cysteine-conjugates are bound to mitochondrial proteins, indicating that they react immediately upon formation (Hayden et al., 1991). Mitochondrial injury has also been shown to be an early event in cisplatin-induced nephrotoxicity (Gordon and Gattone, 1986; Brady et al., 1990).

Inhibitors of GGT and cysteine *S*-conjugate β -lyase have been shown to protect the kidney from haloalkene-induced toxicity in both rats and mice (Anders and Dekant, 1998). The data presented here, in C57BL/6 mice, extend our previous studies in rats showing that GGT activity plays a role in cisplatin-induced nephrotoxicity (Hanigan et al., 1994). There is one report in the literature that conflicts with our data. Ban and coworkers did not find a significant protective effect of acivicin on the renal toxicity of cisplatin in male Swiss OF1 mice (Ban et al., 1994). They quantitated tubule damage in alkaline phosphatase-stained frozen kidney sections from mice sacrificed 72 h after treatment. They do not present data on the level of GGT inhibition; however, in an earlier study assessing the nephrotoxicity of hexachloro-1,3-butadiene in male Swiss OF1 mice, they reported that acivicin inhibited GGT activity by 84% (deCeuriz and Ban, 1990). In our study GGT activity was inhibited by 96%. The difference in the level of GGT activity may account for the different results obtained in the two studies. Additional data obtained recently in our laboratory shows that cisplatin is not nephrotoxic in GGT-knockout mice (Hanigan et al., 2001).

In this study, acivicin and AOAA protected against the nephrotoxicity of cisplatin but had no effect on platinum accumulation in the kidney. These data are in agreement with studies in rats showing that inhibition of GGT blocked the cisplatin-induced nephrotoxicity without altering renal platinum levels (Hanigan et al., 1994, 1996). The data suggest that only a minor fraction of cisplatin is metabolized by GGT and cysteine *S*-conjugate β -lyase, yet is responsible for renal damage. Green and coworkers (1997) have shown in rats that less than 0.01% of a nephrotoxic dose of dichlorovinyl-glutathione is metabolized by GGT and cysteine *S*-conjugate β -lyase to a nephrotoxin. Our data indicate that acivicin and AOAA did not affect the uptake of the parent compound, cisplatin, into the kidney. As noted above, cisplatin has only a low level of toxicity toward nondividing cells. Most of the platinum present in the kidneys, including those treated with acivicin, is likely derived from the binding of the parent compound to cellular nucleophiles. Acivicin blocks the metabolism of the glutathione-cisplatin-conjugate and thereby inhibits its further metabolism to a cysteine-conjugate that is taken up into the cell and converted to a nephrotoxin. But, the cysteine-conjugate may constitute such a small percentage of the total platinum uptake that a reduction in its uptake would be undetectable amid analysis of the total platinum concentration in the kidney.

Conjugation to glutathione and metabolism of a glutathione-cisplatin-conjugate by GGT do not play a role in the tumor toxicity of cisplatin. In fact, cisplatin-glutathione conjugates have been shown to have reduced anti-tumor activity. Conjugation of cisplatin to glutathione-reduced DNA-adduct formation and cytotoxicity in dividing tumor cell lines (Gosland et al., 1996). GGT expression in tumors does not correlate with the patients' response to cisplatin treatment (Hanigan et al., 1998; Nishimura et al., 1998). Studies in nude mice showed GGT accelerated the growth rate and increased the resistance of tumors to cisplatin, rather than enhancing its toxicity (Hanigan et al., 1999). The only report of cysteine *S*-conjugate β -lyase activity in tumor cells shows a very low level of activity in some human renal cell carcinomas (Nelson et al., 1995). In the absence of cysteine *S*-conjugate β -lyase activity, metabolism of a platinum-cysteine-conjugate to a toxic thiol cannot be completed.

Understanding the pathway through which cisplatin is metabolized to a nephrotoxin is essential to the design of new generations of platinum-based chemotherapy drugs. Our data indicate that inhibitors of cysteine *S*-conjugate β -lyase may be useful in significantly reducing the nephrotoxic side effects of cisplatin. Exploiting the different mechanisms of cisplatin-induced nephrotoxicity versus anti-tumor activity may increase the therapeutic index of this clinically important class of chemotherapy agents. The findings in this study provide strong in vivo evidence for the renal activation of cisplatin via a GGT and cysteine *S*-conjugate β -lyase-dependent pathway to a nephrotoxin.

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ABBREVIATIONS:

GGT	γ -glutamyl transpeptidase
acivicin	α -amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid
AOAA	aminooxyacetic acid
BUN	blood urea nitrogen
PLP	pyridoxal 5'-phosphate

References

- Abraham DG, Thomas RJ, and Cooper AJ (1995) Glutamine transaminase K is not a major cysteine *S*-conjugate β -lyase of rat kidney mitochondria: evidence that a high-molecular weight enzyme fulfills this role. *Mol Pharmacol* 48:855–860. [PubMed: 7476916]
- Anders MW and Dekant W (1998) Glutathione-dependent bioactivation of haloalkenes. *Annu Rev Pharmacol Toxicol* 38:501–537. [PubMed: 9597164]
- Ban M, Hettich D, and Huguet N (1994) Nephrotoxicity mechanism of *cis*-platinum(II) diamine dichloride in mice. *Toxicol Lett* 71:161–168. [PubMed: 7909624]
- Brady HR, Kone BC, Stromski ME, Zeidel ML, Giebisch G, and Gullans SR (1990) Mitochondrial injury: an early event in cisplatin toxicity to renal proximal tubules. *Am J Physiol* 258:F1181–F1187. [PubMed: 2159714]
- Cooper AJL (1994) Enzymology of cysteine *S*-conjugate beta-lyases. *Adv Pharmacol* 27:71–113. [PubMed: 8068566]
- Daley-Yates PT and McBrien DCH (1984) Cisplatin metabolites in plasma, a study of their pharmacokinetics and importance in the nephrotoxic and antitumour activity of cisplatin. *Biochem Pharmacol* 33:3063–3070. [PubMed: 6541482]
- Davenport HA (1960) *Histological and Histochemical Techniques*. W.B. Saunders Company, Philadelphia, PA.

- deCeaurre J and Ban M (1990) Role of gamma-glutamyl transpeptidase and beta-lyase in the nephrotoxicity of hexachloro-1,3-butadiene and methyl mercury in mice. *Toxicol Lett* 50:249–256. [PubMed: 1689880]
- Dekant W (1993) Bioactivation of nephrotoxins and renal carcinogens by glutathione *S*-conjugate formation. *Toxicol Lett* 67:151–160. [PubMed: 8451756]
- Dekant W and Henschler D (1999) Organ-specific carcinogenicity of haloalkenes mediated by glutathione conjugation. *J Cancer Res Clin Oncol* 125:174–181. [PubMed: 10235471]
- Dekant W and Vamvakas S (1996) Biotransformation and membrane transport in nephrotoxicity. *Crit Rev Toxicol* 26:309–334. [PubMed: 8726165]
- Elfarra AA, Jakobson I, and Anders MW (1986) Mechanism of *S*-(1,2-dichlorovinyl) glutathione-induced nephrotoxicity. *Biochem Pharmacol* 35:283–288. [PubMed: 2867768]
- Finkelstein MB, Baggs RB, and Anders MW (1992) Nephrotoxicity of the glutathione and cysteine conjugates of 2-bromo-2-chloro-1,1-difluoroethene. *J Pharmacol Exp Ther* 261:1248–1252. [PubMed: 1602387]
- Fowler LM, Foster JR, and Lock EA (1994) Nephrotoxicity of 4-amino-3-*S*-glutathionylphenol and its modulation by metabolism or transport inhibitors. *Arch Toxicol* 68:15–23. [PubMed: 7909430]
- Gordon JA and Gattone VH (1986) Mitochondrial alterations in cisplatin-induced acute renal failure. *Am J Physiol* 250:F991–F998. [PubMed: 3717354]
- Gosland M, Lum B, Schimmelpfennig J, Baker J, and Doukas M (1996) Insights into mechanisms of cisplatin resistance and potential for its clinical reversal. *Pharmacotherapy* 16:16–39. [PubMed: 8700790]
- Green T, Dow J, Ellis MK, Foster JR, and Odum J (1997) The role of glutathione conjugation in the development of kidney tumours in rats exposed to trichloroethylene. *Chem Biol Interact* 105:99–117. [PubMed: 9251723]
- Hanigan MH, Frierson HF Jr, and Taylor PT Jr (1998) Expression of gamma-glutamyl transpeptidase in stage III and IV ovarian surface epithelial carcinomas does not alter response to primary cisplatin-based chemotherapy. *Am J Obstet Gynecol* 179:363–367. [PubMed: 9731839]
- Hanigan MH and Frierson HF Jr (1996) Immunohistochemical detection of gamma-glutamyl transpeptidase in normal human tissue. *J Histochem Cytochem* 44:1101–1108. [PubMed: 8813074]
- Hanigan MH, Gallagher BC, and Taylor PT Jr (1996) Cisplatin nephrotoxicity: Inhibition of gamma-glutamyl transpeptidase blocks the nephrotoxicity of cisplatin without reducing platinum concentrations in the kidney. *Am J Obstet Gynecol* 175:270–274. [PubMed: 8765241]
- Hanigan MH, Gallagher BC, Taylor PT Jr, and Large MK (1994) Inhibition of gamma-glutamyl transpeptidase activity by acivicin in vivo protects the kidney from cisplatin-induced toxicity. *Cancer Res* 54:5925–5929. [PubMed: 7954424]
- Hanigan MH, Gallagher BC, Townsend DM, and Gabarra V (1999) Gamma-glutamyl transpeptidase accelerates tumor growth and increases the resistance of tumors to cisplatin in vivo. *Carcinogenesis* 20:553–559. [PubMed: 10223181]
- Hanigan MH, Lykissa ED, Townsend DM, Ou C, Barrios R, and Lieberman MW (2001) Gamma-glutamyl transpeptidase-deficient mice are resistant to the nephrotoxicity of cisplatin. *Am J Pathol* 159:1889–1894. [PubMed: 11696449]
- Hayden PJ, Ichimura T, McCann DJ, Pohl LR, and Stevens JL (1991) Detection of cysteine conjugate metabolite adduct formation with specific mitochondrial proteins using antibodies raised against haloethane metabolite adducts. *J Biol Chem* 266:18415–18418. [PubMed: 1917965]
- Kim HS, Cha SH, Abraham DG, Cooper AJ, and Endou H (1997) Intranephron distribution of cysteine *S*-conjugate beta-lyase activity and its implication for hexachloro-1,3-butadiene-induced nephrotoxicity in rats. *Arch Toxicol* 71:131–141. [PubMed: 9049049]
- Koob M and Dekant W (1992) Biotransformation of the hexachlorobutadiene metabolites 1-(glutathion-*S*-yl)-pentachlorobutadiene and 1-(cystein-*S*-yl)-pentachlorobutadiene in the isolated perfused rat liver. *Xenobiotica* 22:125–138. [PubMed: 1615703]
- Lash LH, Fisher JW, Lipscomb JC, and Parker JC (2000) Metabolism of trichloroethylene. *Environ Health Perspect* 108(Suppl 2):177–200.

- Lash LH, Qian W, Putt DA, Hueni SE, Elfarra AA, Krause RJ, and Parker JC (2001) Renal and hepatic toxicity of trichloroethylene and its glutathione-derived metabolites in rats and mice: sex-, species-, and tissue-dependent differences. *J Pharmacol Exp Ther* 297:155–164. [PubMed: 11259540]
- Lash LH, Sausen PJ, Duescher RJ, Cooley AJ, and Elfarra AA (1994) Roles of cysteine conjugate *β*-lyase and *s*-oxidase in nephrotoxicity: studies with *s*-(1,2-dichlorovinyl)-L-cysteine and *s*-(1,2-dichlorovinyl)-L-cysteine sulfoxide. *J Pharmacol Exp Ther* 269:374–383. [PubMed: 8169843]
- Lau SS and Monks T (1990) The in vivo disposition of 2-bromo-[14C]hydroquinone and the effect of gamma-glutamyl transpeptidase inhibition. *Toxicol Appl Pharmacol* 103:121–132. [PubMed: 1969181]
- Madias NE and Harrington JT (1978) Platinum nephrotoxicity. *Am J Med* 65:307–314. [PubMed: 99034]
- Mehta PK and Christen P (2000) The molecular evolution of pyridoxal-5'-phosphate-dependent enzymes. *Adv Enzymol Relat Areas Mol Biol* 74:129–184. [PubMed: 10800595]
- Mistry P, Lee C, and McBrien DC (1989) Intracellular metabolites of cisplatin in the rat kidney. *Cancer Chemother Pharmacol* 24:73–79. [PubMed: 2731314]
- Monks TJ, Hight RJ, and Lau SS (1988) 2-Bromo-(diglutathion-*S*-yl)hydroquinone nephrotoxicity: physiological, biochemical, and electrochemical determinants. *Mol Pharmacol* 34:492–500. [PubMed: 3173333]
- Nelson JA, Pan BF, Swanson DA, and Elfarra AA (1995) Cysteine conjugate beta-lyase activity in human renal carcinomas. *Cancer Biochem Biophys* 14:257–263. [PubMed: 7767899]
- Nishimura T, Newkirk K, Sessions RB, Andrews PA, Trock BJ, Rusmussen AA, Montgomery EA, Bischoff EK, Hanigan MH, and Cullen KJ (1998) Association between expression of glutathione-associated enzymes and response to platinum-based chemotherapy in head and neck cancer. *Chem-Biol Interact* 111–112:187–198.
- Sadzuka Y, Shimizu Y, Takino Y, and Hirota S (1994) Protection against cisplatin-induced nephrotoxicity in the rat by inducers and an inhibitor of glutathione *S*-transferase. *Biochem Pharmacol* 48:453–459. [PubMed: 8068032]
- Stevens JL (1985) Isolation and characterization of a rat liver enzyme with both cysteine conjugate beta-lyase and kynureninase activity. *J Biol Chem* 260:7945–7950. [PubMed: 4008484]
- Stevens JL, Robbins JD, and Byrd RA (1986) A purified cysteine conjugate beta-lyase from rat kidney cytosol. Requirement for an alpha-keto acid or an amino acid oxidase for activity and identity with soluble glutamine transaminase K. *J Biol Chem* 261:15529–15537. [PubMed: 3782077]
- Trimmer EE and Essigmann JM (1999) Cisplatin. *Essays Biochem* 34:191–211. [PubMed: 10730196]

Fig. 1.

Structure of cisplatin and nephrotoxic haloalkenes and proposed metabolism of cisplatin to a nephrotoxin. A, the structure of cisplatin and two nephrotoxic haloalkenes: trichloroethene and hexachloro-1,3-butadiene. B, the proposed pathway for the metabolism of cisplatin to a nephrotoxin. The pathway is based on the metabolic pathway through which the haloalkenes are activated to nephrotoxins. The first step is the conjugation to glutathione, followed by the cleavage by GGT to a cysteinyl-glycine conjugate, the cleavage by aminopeptidase to a cysteine *S*-conjugate, and the β -elimination by cysteine *S*-conjugate β -lyase to an unstable thiol that yields a highly reactive electrophile.

Fig. 2. BUN values 5 Days after treatment. Mice were pretreated with saline, acivicin, or AOAA followed by an injection of either saline or 15 mg/kg cisplatin. Five days after treatment blood was collected and analyzed for BUN. Data are shown for mice pretreated with saline (solid bar), acivicin (stippled bar), and AOAA (striped bar) and treated with saline (three left bars) or cisplatin (three right bars). The bars indicate the mean of each group $\pm$ S.D. a, the mean differed significantly from all other groups ($p < 0.05$).

Fig. 3. Effect of pretreatment with acivicin or AOAA on cisplatin-induced proximal tubule damage. Hematoxylin and eosin stained sections of kidneys from mice 5 days after treatment. Shown are representative sections from the saline treatment group (A), the cisplatin treatment group (B), the acivicin cisplatin treatment group (C), and the AOAA cisplatin treatment group (D). Arrows indicate protein casts.

TABLE 1
Protective effect of acivicin and AOAA on mortality of cisplatin treatment

Mice were pretreated with saline, acivicin, or AOAA followed by an injection of either saline or 15 mg/kg cisplatin. The mice were monitored for 5 days. The acute mortality of the treatment was determined by the number of mice that died within 5 days.

Pretreatment	Mortality	
	Saline	Cisplatin
	<i>No. dead mice/total</i>	
Saline	0/8	6/11 ^a
Acivicin	0/8	0/8
AOAA	0/7	0/7

^aSignificantly different from all other groups $p < 0.04$.

TABLE 2
Effect of treatment on mouse weight

Mice were pretreated with saline, acivicin, or AOAA followed by an injection of either saline or 15 mg/kg cisplatin. Five days after treatment, all surviving mice were weighed. The weight change for each mouse was calculated as the percentage of the weight before treatment. The average percent weight change was calculated for each group.

Pretreatment	Weight Change	
	Saline	Cisplatin
	%	
Saline	-0.1 ± 2	-24.3 ± 5^a
Acivicin	-0.9 ± 1	-20.4 ± 3^a
AOAA	$+3.6 \pm 1$	-17.1 ± 3^a

^aSignificantly different from the saline treatment group, acivicin treatment group, and AOAA treatment group ($p < 0.05$), but no significant difference among the three cisplatin-treated groups.

TABLE 3
Protective effect of acivicin and AOAA on cisplatin-induced renal toxicity

Mice were pretreated with saline, acivicin, or AOAA followed by an injection of either saline or 15 mg/kg cisplatin. Five days after treatment, all surviving mice were sacrificed, and the right kidney from each mouse was fixed in Bouin's fixative, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Protein casts, which are indicators of renal damage, were counted. The average number of protein casts per 10× field was computed for each group.

Pretreatment	Number of Protein Casts/10× Field	
	Saline	Cisplatin
Saline	0 ± 0	9.8 ± 1.1 ^a
Acivicin	0 ± 0	1.2 ± 0.9 ^a
AOAA	0 ± 0	0 ± 0

^aSignificantly different from all other treatment groups ($p < 0.05$).

TABLE 4
Effect of acivicin and AOAA on platinum levels in the kidney 5 days after treatment with cisplatin

Mice were pretreated with saline, acivicin, or AOAA followed by an injection of 15 mg/kg cisplatin. Five days after treatment, all surviving mice were sacrificed, and the left kidney from each mouse was analyzed for platinum content by atomic absorption spectroscopy. No significant difference was found among cisplatin-treated groups.

Pretreatment	Platinum Level 5 days after Cisplatin Treatment
	<i>μg Pt/mg Kidney</i>
Saline	8.0 ± 1.8
Acivicin	8.6 ± 1.5
AOAA	7.0 ± 0.4