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Metabolism of Cisplatin to a Nephrotoxin

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During the past decade we have published a series of papers detailing our discovery that cisplatin is bioactivated to a nephrotoxin^{1–8}. In July of this year, Wainford and coworkers published a paper in *Toxicology* entitled “Cisplatin nephrotoxicity is mediated by gamma glutamyltranspeptidase, not via a C-S lyase governed biotransformation pathway”⁹. In this publication, Wainford and coworkers state that they have repeated several of our *in vivo* and *in vitro* experiments and disagree with our conclusions regarding the final two steps in the metabolism of cisplatin to a nephrotoxin.

Our data have shown that a glutathione *S*-conjugate of cisplatin is cleaved by γ -glutamyl transpeptidase (GGT) to a cysteinyl-glycine *S*-conjugate. Dipeptidase(s) catalyze(s) further cleavage to a cysteine *S*-conjugate. Both our *in vivo* and *in vitro* data have shown that the platinum-cysteine *S*-conjugate can be converted to a toxic, highly reactive platinum-thiol by any of several enzymes that catalyze the cysteine *S*-conjugate β -lyase (C-S lyase) reactions.

Wainford and coworkers treated both rats and mice with acivicin, an inhibitor of GGT, and showed that inhibition of GGT protected against cisplatin induced renal toxicity. These data replicate our previously published findings. They also treated rats and renal cell cultures with inhibitors of dipeptidases. Rats and isolated renal cells were treated with either bestatin (inhibiting 90% of aminopeptidase N) or with L-phenylalanylglycine which inhibits renal dipeptidase. Neither inhibitor blocked the nephrotoxicity of cisplatin. Wainford et al interpreted these results as indicating that dipeptidases are not involved in the metabolism of cisplatin to a renal toxin. However, both of these enzymes have been shown to cleave cysteinyl-glycine conjugates (other enzymes in this class, such as leucine aminopeptidase¹⁰, may also cleave these conjugates). Since these enzymes can act redundantly, inhibiting either enzyme alone does not block dipeptidase activity. Thus, the experimental data presented by Wainford et al. do not adequately address their hypothesis. Their conclusion that dipeptidase(s) have no role in the bioactivation of cisplatin is unwarranted.

The authors also imply that glutamine transaminase K (GTK) is the only enzyme that catalyzes the cysteine *S*-conjugate β -lyase reaction, whereas to date eleven different mammalian enzymes have been shown to catalyze cysteine *S*-conjugate β -lyase reactions *in vitro* with halogenated cysteine *S*-conjugates, most notably mitochondrial aspartate aminotransferase

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(mitAspAT)¹¹. The authors state that in one of our studies (ref. 8) we overexpressed mitAspAT as a strategy to increase GTK levels. This statement is a misrepresentation of our experiments. The authors may not be aware that mitAspAT is a C-S lyase in its own right. What we have shown is that overexpression of either GTK or mitAspAT in renal cells in culture increases the bioactivation of cisplatin to a nephrotoxin^{7,8}.

Wainford and co-workers mistakenly take issue with published data on the renal toxicity of cisplatin in aminooxyacetic acid (AOAA)-treated rats that they attribute to us. We have never published data on AOAA and cisplatin in rats and to our knowledge no other group has reported such data. We have shown that AOAA protects C57BL/6 male mice from cisplatin-induced renal toxicity. Wainford and co-workers claim to have repeated our mouse experiments. However, they used considerably older mice. They also reduced the concentration of cisplatin, altered the dosing schedule of AOAA, failed to include a control group of mice treated with AOAA alone, and reduced the time course of the experiment. It is not clear to us which if any of these alterations in our protocol led to the results that differed from those that we obtained.

Wainford and coworkers treated both rat and human proximal tubular cells in culture with cisplatin. They report that they did not observe any protective effect of acivicin or AOAA against cisplatin-induced toxicity in these cells. Their data are in direct contrast to our data with LLC-PK₁ cells. Both acivicin and AOAA inhibit essential enzymes in the cell and prolonged exposure is toxic. Wainford and coworkers pretreated the cells with acivicin or AOAA, included the inhibitors during a 2 hour exposure to cisplatin and continued to treat the cells with these inhibitors for the entire 24 hour or 48 hour experiment. The toxicity of prolonged high doses of these inhibitors likely masked any protective effect that the inhibitors had against cisplatin toxicity. In contrast, we pretreated cells with these inhibitors and maintained the inhibitors only during the 3 hour cisplatin treatment. We then removed both the inhibitor and the cisplatin and measured cell viability at 72 hours.

Finally, Wainford and co-workers reference a previous publication from their laboratory for the method used to assay GTK¹². In the previous publication they describe the assay but do not include any reference for it, indicating that they developed it. The assay was, in fact, developed by Dr. Arthur Cooper who discovered GTK¹³.

Wainford and coworkers fail to offer any hypothesis regarding the metabolism of the platinum cysteinyl-glycine *S*-conjugate to a nephrotoxin. Their data do not disprove our hypothesis that a C-S lyase metabolizes the cysteine *S*-conjugate of cisplatin to a nephrotoxin. Our data in multiple model systems provide strong support for our hypothesis that the bioactivation of cisplatin to a renal toxin proceeds via the conversion of the platinum-cysteinyl-glycine *S*-conjugate to the cysteine *S*-conjugate by dipeptidase(s) followed by a cysteine *S*-conjugate β -lyase reaction.

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