Cisplatin-DNA adduct formation in kidneys of cisplatin-exposed rats protected from nephrotoxicity by acivicin or glutathione

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Abstract: **AIM** In order to examine the mechanisms by which these compounds prevent the development of nephrotoxicity, we investigated cisplatin-DNA adduct formation in kidneys of rats given either cisplatin alone or pretreatments with acivicin or GSH. **METHODS** Rats were given cisplatin 6 $mg \cdot kg^{-1}$ body weight by tail vein injection and sacrificed 5 days later. **RESULTS** Pretreatment with acivicin 10 $mg \cdot kg^{-1}$ body weight 2.5 h before the cisplatin completely blocked cisplatin-induced nephrotoxicity, as determined by blood urea nitrogen (BUN) and serine creatinine, and reduced renal DNA adducts by 17.1% (not statistically significant). Pretreatment with GSH 500 $mg \cdot kg^{-1}$ body weight significantly reduced nephrotoxicity and lowered cisplatin-DNA adduct levels by 45.2% ($P < 0.05$). In addition, a weakly-positive linear relationship was observed between cisplatin-DNA adducts and BUN level ($r = 0.47$, $P = 0.03$), and adducts and serum creatinine level ($r = 0.50$, $P = 0.02$). **CONCLUSION** The associations observed between cisplatin-DNA adduct levels and these nephrotoxic end points suggest that cisplatin-DNA adducts or their correlates be only weakly associated with kidney damage. The lack of strong associations here supports the conclusion that cisplatin-DNA adducts are not the major cause of cisplatin-induced kidney toxicity.

Key words: DNA adducts; cisplatin; glutathione; toxicity; acivicin

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cis - Diamminedichloroplatinum (Ⅱ) (cis-platin) is widely-used for the treatment of testicular and ovarian cancer, but dosing is limited in a clinical setting by the induction of renal damage[1-3]. Cisplatin-DNA adduct formation is considered to be associated with cisplatin-induced toxicity[4-7] as well as therapeutic effects[8-12], but there is no direct evidence linking cisplatin-induced nephrotoxicity with renal cisplatin-DNA adducts. Furthermore the mechanism of cisplatin-induced renal damage is not well understood. In adult rat kidney, the major site for cisplatin toxicity is the proximal tubular cells, which are essentially quiescent, suggesting that replication on a damaged template be not the primary toxic mechanism.

Previous studies have shown that acivicin, an irreversible inhibitor of γ-glutamyl transpeptidase (GGT), completely blocks cisplatin-induced nephrotoxicity[13,14] while excess glutathione (GSH), a competitive inhibitor of GGT, only partially blocks this toxicity[13,14]. High levels of GGT are expressed on the surface of the renal proximal tubular cells and GGT cleaves the γ-glutamyl bonds of substrates including GSH and GSH conjugates[15,16]. Previous studies[13,14] have indicated that GGT is required for renal damage and suggest that GGT cleavage of cisplatin-GSH conjugates be responsible for the renal damage. The present study was designed to further elucidate the mechanism of cisplatin-induced renal damage by examining the role of cisplatin-DNA adducts in nephrotoxicity in the same animals that were previously studied for kidney damage with and without acivicin and GSH[14].

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1 MATERIALS AND METHODS

1.1 Animals and treatment protocol

The animal treatment protocol has been described in detail[14]. The protocol used was approved by the University of Virginia Medical School Animal Care and Use Committee according to USPH Guidelines for Care and Use of Laboratory Animals. In brief, male Sprague-Dawley rats (275 – 300) g were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Inc., Mundelein, IL). A 24-gauge Teflon polytetrafluoroethylene 0.75-inch catheter (Critikon, Inc., Tampa, FL) was placed in the tail vein and the catheter was capped with intermittent injection caps and flushed with 0.2 mL heparinized saline solution. The animals were divided into five groups (7 – 8 rats for each group) and injected with the following combinations of chemicals: group A, saline solution; group B, acivicin (10 mg·kg^{-1} body weight); group C, cisplatin (6 mg·kg^{-1}); group D, acivicin (10 mg·kg^{-1}) followed 2.5 h later with cisplatin (6 mg·kg^{-1}); and group E, GSH (500 mg·kg^{-1}) followed 30 min later with cisplatin (6 mg·kg^{-1}). Five days later, animals were weighed and sacrificed. Blood was collected by heart puncture. Kidneys were removed and stored at – 80℃.

1.2 Serum assays and atomic absorbance spectrometry(AAS)

Blood urea nitrogen(BUN) and serum creatinine levels were measured with the diagnostic colorimetric end point kits from Sigma, as described previously[14]. Tissue platinum levels in kidneys were determined by AAS as described previously[13].

1.3 Cisplatin-DNA adduct determination

Nuclear DNA was extracted using CsCl buoyant density gradient centrifugation and quantified absorbance at 260 nm as previously described[17]. One sample from group C was lost during the DNA extraction. Cisplatin-DNA adducts were determined in kidney DNA using dissociation enhanced lanthanide fluoroimmunoassay (DELFIA), an adaption of the enzyme-linked immunosorbent assay as previously described[18,19].

1.4 Statistical analysis

One way ANOVA, followed by Student-Newman-Keuls multiple comparison, was performed for the data in Tab 1 using the SigmaStat program. Linear regression analysis in Fig 1 was performed using the GraphPad Prism program.

2 RESULTS

This study was designed to investigate the relationship between cisplatin-DNA adduct formation and renal damage in kidneys of rats given cisplatin with and without acivicin and/or GSH, compounds known to modulate cisplatin-induced renal toxicity in the rats. Modulation of renal toxicity was previously measured in these same animals[13,14] and values for BUN, serum creatinine and kidney tissue platinum are shown in Tab 1 for comparison. The cisplatin-DNA adduct measurements reported here were obtained subsequently from kidney tissues stored frozen after the completion of the previously-published experiments[13,14]. In kidneys of rats treated with cisplatin alone (Tab 1, group C), the cisplatin-DNA adduct level was (43.8 ± 12.8) nmol·g^{-1} DNA. In the rats given acivicin 2.5 h prior to cisplatin (Tab 1, group D), the kidney cisplatin-DNA adduct level was (36.3 ± 7.2) nmol·g^{-1} DNA, and this was not a significant decrease compared to the rats treated with cisplatin alone ($P > 0.5$). In the rats treated with GSH for 30 min prior to cisplatin exposure (Tab 1, group E), the renal cisplatin-DNA adduct level of (24.0 ± 5.1) nmol·g^{-1} DNA was significantly decreased as compared to group C ($P < 0.05$, Tab 1). Tab 1 also shows no difference in the kidney tissue platinum levels in the three cisplatin-exposed groups.

To define the relationship between cisplatin-DNA adduct and renal functional damage, the values of BUN or serum creatinine from each indivi- dual animal of the groups C, D and E were plotted against the cisplatin-DNA adduct level. Linear regression analysis (Fig 1) shows a weak, positive correlation between BUN levels and DNA adducts (Fig 1A, $r = 0.47$, $P = 0.03$) and between

Tab 1. Cisplatin-DNA adduct levels in rats[a] 5 d after exposure to cisplatin with or without acivicin or GSH pretreatment(intravenous injection). For comparison the table contains previously-published data for BUN, serum creatinine and kidney tissue platinum determined in the same animals

Group	Treatment /mg·kg^{-1}	BUN[b] /g·L^{-1}	Serum creatinin[b] /mg·L^{-1}	Kidney platinum[c] /μg·g^{-1} tissue	Kidney cisplatin-DNA adduct[a] /nmol·g^{-1} DNA
A	Saline	0.22 ± 0.09	5.71 ± 1.67	ND[d]	ND
B	Acivicin 10	0.22 ± 0.05	4.06 ± 0.81	ND	ND
C	Cisplatin 6	1.32 ± 0.34*	22.46 ± 4.89*	8.17 ± 0.16	43.8 ± 12.8
D	Cisplatin 6 + acivicin 10	0.31 ± 0.17	5.75 ± 1.50	7.7 ± 1.3	36.3 ± 7.2
E	cisplatin 6 + GSH 500	0.46 ± 0.42	11.35 ± 6.02*	7.7 ± 1.2	24.0 ± 5.1#

a: values are mean ± *s* of 7 to 8 rats. b: reported previously in 14. c: reported previously in 13. d: ND = not detectable. * $P < 0.05$, compared with the other groups; # $P < 0.05$, compared with group C but not group D.

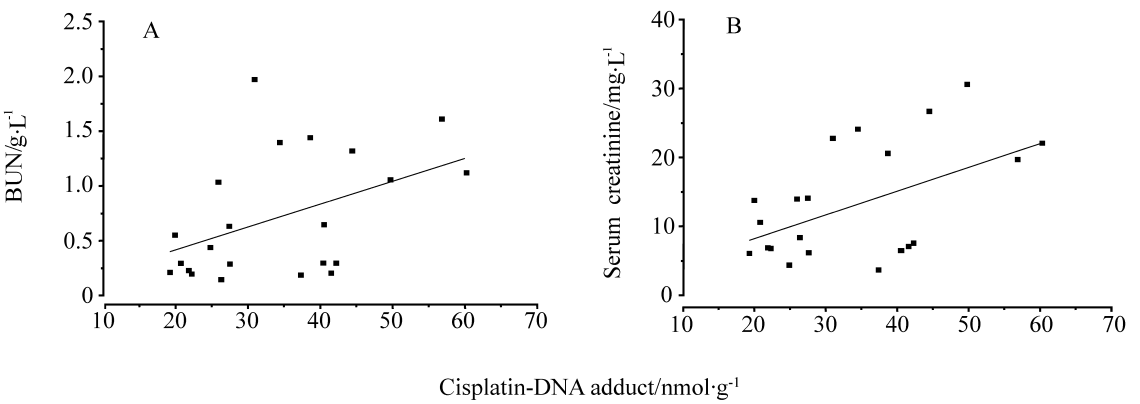

Fig 1. Linear regression analysis of the correlation between kidney cisplatin-DNA adducts and BUN (A) or serum creatine (B) levels. DNA adduct values are from the cisplatin-exposed rats (groups C, D and E, Tab 1) using GraphPad Prism. For Fig 1A, $r = 0.47$, $P = 0.03$. For Fig 1B, $r = 0.50$, $P = 0.02$.

serum creatinine levels and DNA adducts (Fig 1B, $r = 0.50$, $P = 0.02$).

3 DISCUSSION

This study demonstrates that acivicin pretreatment does not alter the formation of cisplatin-DNA adducts in kidneys of rats given cisplatin 6 mg·kg^{-1} body weight and sacrificed 5 d later. However, with acivicin pretreatment the BUN and serum creatinine levels were essentially normal. This would suggest that cisplatin-induced nephrotoxicity be independent of cisplatin-DNA adduct formation. Previous studies using acivicin[13,14] have indicated that cisplatin-induced nephrotoxicity requires GGT. Since acivicin binds cell-surface GGT non-competitively to inhibit GGT irreversibly and reduces cisplatin-induced nephrotoxicity, acivicin may have the potential to eliminate cisplatin-induced renal toxicity without significantly compromising the therapeutic effects of the drug. This hypothesis is plausible since the clinical efficacy of cisplatin is considered to be dependent upon factors involving genotoxicity, including extent of DNA adduct formation[8-12] and removal[20-22].

In the case of the GSH pretreatment, the very large dose of this scavenger not-only partially blocks nephrotoxicity by competitively occupying cell surface GGT, but also results in less cisplatin binding to DNA. It is possible that the large excess of GSH produces a non-toxic GSH-cisplatin complex, which reduces the cisplatin concentration available to bind to the DNA. This hypothesis will be the subject of further investigations.

Since the kidney tissue levels of cisplatin were similar in all three exposed groups it would appear that the differences in adduct levels and nephrotoxicity were not due to treatment-specific differences in amounts of available cisplatin. The associations observed between cisplatin-DNA adduct levels and the nephrotoxic end points, BUN and serum creatinine, suggest that cisplatin-DNA adducts are weakly associated with kidney damage. Since no dose-response relationship and time-course relationship were performed in this study, it is very difficult to determine if a stronger association between cisplatin-DNA adducts and kidney damage exists. This question will be investigated in the future studies.

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阿西维辛或谷胱甘肽降低顺铂引起的肾脏毒性和顺铂-DNA 加合物在鼠肾脏中的水平

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摘要：目的　为了了解阿西维辛(acivicin)和 GSH 预防肾脏毒性的机制，研究了顺铂-DNA 加合物在大鼠肾脏中的水平。**方法**　顺铂(6 mg·kg^{-1})从尾静脉注入大鼠，5 d 后处死。其他两组动物在给顺铂前 2.5 h，给予阿西维辛或者 GSH。测量顺铂-DNA 加合物在肾脏中的浓度、血中尿素氮(BUN)和丝氨酸肌酸的浓度。**结果**　在给顺铂前 2.5 h，给 10 mg·kg^{-1}阿西维辛完全阻断了顺铂引起的肾脏毒性。具体表现是血氮和肌酸浓度降低，DNA 加合物减少了 17.1%($P < 0.05$)。在给顺铂前，给 500 mg·kg^{-1} GSH，肾脏毒性和顺铂-DNA 加合物水平均显著性减低($P < 0.05$)。另外，在 DNA 加合物和血氮之间存在着一个弱正相关关系。**结论**　DNA 加合物在顺铂引起的肾脏毒性中引了一些作用。但是 DNA 加合物和血氮之间的弱相关关系提示 DNA 加合物与肾脏毒性只有较弱的联系，在顺铂引起的肾脏毒性中不是主要因素。

关键词： DNA 加合物；顺铂；谷胱甘肽；毒性；阿西维辛

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