EFFECTS OF ZINC GLUCONATE ON NEPHROTOXICITY AND GLUTATHIONE METABOLISM DISORDER INDUCED BY *CIS*-PLATIN IN MICE

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SUMMARY

To examine the effects of zinc gluconate (ZG) on the nephrotoxicity of *cis*-platin (CDDP), changes in renal function and glutathione metabolism were investigated in mice. Fifty mice were randomly divided into five equal groups: controls, CDDP group and three ZG plus CDDP groups; the dose of ZG was 100 mg, 140 mg and 180 mg/kg respectively. Mice were sacrificed after 48 hours of CDDP (9 mg/kg) treatment; the levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were measured, the glutathione (GSH) and malondialdehyde (MDA) content and the activities of glutathione peroxidase (GHS-PX) and catalase (CAT) in whole blood and renal tissue were assayed. Compared to the control group, there were significant increases in Cr, BUN and MDA, and decreases in the activities of GSH-PX in the CDDP group in whole blood and renal tissue. Preadministration of ZG at the doses of 140 mg/kg and 180 mg/kg significantly increased the concentration of GSH in whole blood, and decreased the levels of Cr and BUN, and the MDA content in whole blood and renal tissue, compared with the CDDP group. We conclude that preadministration of ZG might improve renal function and glutathione metabolism and reduce the nephrotoxicity of *cis*-platin.

KEY WORDS

zinc gluconate, *cis*-platin, nephrotoxicity, glutathione, glutathione peroxidase, malondialdehyde, catalase, mouse

INTRODUCTION

Cis-platin (*cis*-diamminedichloroplatinum, CDDP) is an effective antitumour drug widely used against several types of malignant tumour; however, the clinical application of CDDP is limited by its nephrotoxicity and its mechanism of action remains unknown /1/. Previous studies have found that some of the side effects of CDDP can be prevented by preadministration of zinc gluconate (ZG) /2/. The effects of ZG on changes in renal function and glutathione metabolism induced by CDDP were investigated in mice.

MATERIALS AND METHODS

Zinc gluconate (ZG) (99.24% pure) was kindly donated by the Fifth Pharmaceutical Factory of Chongqing, China. CDDP was purchased from Qilu Pharmaceutical Factory of Jinan, China. All other chemicals were of analytical grade.

Mice, Kunming strain, weighing 21±2 g, were purchased from the Experimental Animal Center of the Third Military Medical University.

Fifty mice were randomly divided into five equal groups: a control group, CDDP group, ZG (100 mg/kg) with CDDP group, ZG (140 mg/kg) with CDDP group and ZG (180 mg/kg) with CDDP group. The mice were given 0.9% NaCl solution (control group and CDDP group) or ZG (100 mg, 140 mg or 180 mg per kilogram) (ZG groups) p.o. once a day for seven consecutive days. On the sixth day, the mice were injected i.p. with 0.9% NaCl solution in the control group or CDDP (9 mg per kilogram) in the other groups, and were sacrificed after 48 hours of CDDP treatment. Blood samples were taken from the orbit and renal tissue was taken for biochemical analysis.

The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were measured with H7150 auto biochemical analytical instrument; the concentrations of glutathione (GSH) and malondialdehyde (MDA) and the glutathione peroxidase (GSH-Px) and catalase (CAT) activities were assayed in whole blood and renal tissue.

The results are shown as means ± SD. Student's t-test was used for statistical analysis.

RESULTS

The levels of serum creatinine and blood urea nitrogen in the CDDP group were significantly higher than in the control group ($p<0.01$). In the groups preadministered with zinc gluconate, the levels of Cr and BUN were significantly decreased compared to the CDDP group ($p<0.05$, $p<0.01$), but still higher than in the control group ($p<0.01$); there were no significant differences between the ZG groups ($p>0.05$) (Table 1).

TABLE 1

The effects of ZG on the changes of Cr and BUN induced by CDDP in mice

Group	Cr ($\mu mol.l^{-1}$)	BUN ($mmol.l^{-1}$)
Control	39.80±6.47	7.88±0.74
CDDP	82.87±14.37**	26.18±9.68**
ZG (100 mg) + CDDP	62.34±14.26**##	16.43±8.87*#
ZG (140 mg) + CDDP	61.16±9.43**##	11.49±7.52##
ZG (180 mg) + CDDP	59.83±10.61**##	11.33±6.47##

Mean ± SD, n=10
*p<0.05, ** p<0.01 compared to control group
#p<0.05, ## p<0.01 compared to CDDP group

The concentration of glutathione in blood in the CDDP group was markedly lower than in the control group ($p<0.01$). Preadministration of ZG at the dose of 140 mg/kg or 180 mg/kg significantly increased the level of glutathione compared with the CDDP group ($p<0.01$); the GSH-Px activity in blood in the CDDP group and the ZG groups were significantly lower than in the control group ($p<0.01$). There was no significant difference between the CDDP group and the ZG groups ($p>0.05$). The change in MDA concentration in all the experimental groups was similar to the change in GSH. There was no change in CAT activity in all groups ($p>0.05$) (Table 2).

TABLE 2

The effects of ZG on the changes of GSH, GSH-Px, CAT and MDA induced by CDDP in blood of mice

Group	GSH (mg.g^{-1}Hb)	GSH-Px (U.g^{-1}Hb)	CAT (U.g-1Hb)	MDA (nmol.l^{-1})
Control	2.22±0.50	35.92±4.95	145.53±21.09	164.19±40.87
CDDP	1.69±0.22*	20.06±8.58*	132.03±24.14	351.44±95.52*
ZG(100)+CDDP	1.86±0.26	21.69±7.84*	138.27±25.23	290.25±86.13
ZG(140)+CDDP	2.02±0.27##	22.67±7.95*	142.87±21.29	251.26±86.13*
ZG(180)+CDDP	2.19±0.40##	25.70±7.07*	143.12±23.75	210.37±70.68##

Mean ± SD, n=10
*p<0.01compared to the control group
#p<0.05, ##p<0.01 compared to the CDDP group

In renal tissue, the concentration of GSH in the CDDP group was decreased compared to the control group ($p<0.05$). Preadministration with different doses of ZG (100 mg, 140 mg or 180 mg per kilogram) did not significantly change the levels of GSH; the activities of GSH-Px and CAT were not different in all the experimental groups ($p>0.05$). The MDA content in the CDDP group was significantly higher than in the control group. Pretreatment with ZG at the dose of 140 mg or 180 mg per kilogram significantly decreased MDA content in renal tissue compared to the CDDP group ($p<0.05$) (Table 3).

DISCUSSION

It is well known that *cis*-platin administration leads to severe renal damage in both experimental studies and clinical practice, but the mechanism of nephrotoxicity has not been completely clarified /1,3/. Results of previous studies have suggested that the manifestations of CDDP toxicity are similar to the toxicity of other heavy metal elements. Kagi and Vallee /4/ reported that preadministration of low doses of cadmium, zinc or copper protected against the toxic effects of heavy metals in horses. Naganuma *et al.* noted that bismuth subnitrate

TABLE 3

The effects of zinc gluconate on the changes of GSH, GSH-Px, CAT and MDA induced by CDDP in renal tissue of mice

Group	GSH ($mg.g^{-1}Prot.$)	GSH-Px ($U.g^{-1}Prot.$)	CAT ($U.g^{-1}Prot.$)	MDA ($nmol.g^{-1}Prot.$)
Control	1.71±0.35	33.00±9.87	27.80±7.66	169.4±36.8
CDDP	1.35±0.33*	25.60±7.91	22.72±4.25	230.7±51.7**
ZG(100)+CDDP	1.58±0.34	24.94±10.60	23.03±7.00	201.2±30.0
ZG(140)+CDDP	1.64±0.30	32.88±12.50	25.97±4.93	189.0±19.1#
ZG(180)+CDDP	1.67±0.44	27.91±12.60	24.00±5.47	191.9±21.3#

Mean ± SD, n=10
*$p<0.01$, **$p<0.01$ compared to the control group
#$p<0.05$ compared to the CDDP group

(BSN) might decrease the toxicity of *cis*-platin by inducing the synthesis of metallothionein (MT) /5/. We found that pretreatment with ZG decreased toxicity and bone marrow inhibition by CDDP /2/. This suggests that ZG may be used as an attenuating agent to reduce the nephrotoxicity of CDDP.

The present study found that preadministration of ZG significantly decreased the increase in levels of serum creatinine and blood urea nitrogen caused by CDDP. ZG, at the doses of 140 mg and 180 mg per kilogram, maintained the glutathione level and glutathione peroxidase activity, and reduced the MDA content in whole blood and renal tissue. These findings suggest that preadministration of ZG should have a preventive effect on CDDP-induced nephrotoxicity.

Zinc is an important trace element in the body which is necessary for many enzymes in their synthesis or as a cofactor. Glutathione peroxidase (GSH-Px), which needs zinc, inactivates lipid peroxide generated during metabolism and so protects cellular membranes and proteins. An interrelationship between lipid peroxidation and the nephrotoxicity of CDDP has not been demonstrated, but several authors have hypothesized that lipid peroxidation contributes to the renal damage by CDDP /3,5/. Zhang *et al.* /6/ reported that lipid

peroxide increased and glutathione was depleted in renal tissue on incubation of renal cortical slices with 2 nM CDDP. These findings are similar to our results *in vivo*. The findings that preadministration of ZG significantly increased the concentration of glutathione and the activity of GSH-Px in whole blood and markedly decreased MDA content in whole blood and renal tissue imply that ZG might improve glutathione metabolism and antagonize the toxicity of CDDP.

In summary, preadministration of ZG might ameliorate the nephrotoxicity of CDDP by decreasing the levels of serum creatinine and blood urea nitrogen, increasing the level of glutathione and the activity of glutathione peroxidase in whole blood and reducing the MDA content in whole blood and renal tissue.

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