Protective Effect of *N*-Benzyl-D-glucamine Dithiocarbamate against *cis*-Diamminedichloroplatinum-Induced Toxicity in Gastrointestinal Tract and Bone Marrow in Rats

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Received May 15, 1990

The protective effect of *N*-benzyl-D-glucamine dithiocarbamate (BGD) against gastrointestinal and bone marrow toxicities produced by *cis*-diamminedichloroplatinum (DDP) injection in rats was studied. Rats were injected i.p. with BGD (2 mmol/kg) immediately after i.v. injection of DDP (20 μmol/kg). A scanning electron micrograph of the jejunum after DDP treatment showed damage in the villi, and that BGD protected the DDP-induced jejunal damage. BGD treatment also had a protective effect against DDP-induced diarrhea. BGD significantly reversed the reduction in maltase and sucrase activities of jejunal mucosa of rats treated with DDP. Platinum (Pt) concentrations in the gastrointestine as well as in the kidney and liver after DDP injection decreased following BGD treatment. The reduction of leukocytes following DDP injection returned to control values after BGD treatment. Biliary and urinary excretions of Pt after DDP injection was remarkably increased by BGD treatment. The results of this study indicated that the injection of BGD to rats treated with DDP can effectively remove Pt from the body through biliary and urinary excretions, resulting in protection of the gastrointestinal and bone marrow toxicities induced by DDP treatment.

Keywords *cis*-diamminedichloroplatinum; *N*-benzyl-D-glucamine dithiocarbamate; chelate effect; gastrointestinal toxicity; bone marrow toxicity; excretion; distribution

cis-Diamminedichloroplatinum (DDP) is a highly effective antitumor drug widely used for the therapy of various human tumors.[1–5] Renal pathology,[1,6] the major side effect, and a variety of other side effects[7] are frequently observed with this drug. A number of sulfur nucleophiles, such as sodium thiosulfate,[8] cysteamine,[9] 5,2-(3-aminopropylamino)ethylphosphorothioic acid (WR2721),[10] 2,3-dimercaptopropanol,[11] 2,3-dimercaptosuccinic acid,[11] and glutathione,[12] have been studied as antidotes to DDP-induced nephrotoxicity with no or slight inhibition of its antitumor effect. The ability of diethyldithiocarbamate to control the nephrotoxicity of DDP has been reported by Borch and his collaborators.[13–17] Some studies have indicated that various dithiocarbamate derivatives, such as *N*-methyl-D-glucamine dithiocarbamate,[18] dimethyldithiocarbamate, dihydroxyethyldithiocarbamate, sarcosine dithiocarbamate, and iminodiacetic acetate dithiocarbamate,[19–21] are able to control DDP-induced nephrotoxicity. More recently, we reported that *N*-benzyl-D-glucamine dithiocarbamate (BGD), a new dithiocarbamate derivative, had a highly protective effect against DDP-induced nephrotoxicity without inhibition of its antitumor effect in rats.[22]

The purpose of the present study was to determine whether BGD decreased or prevented the side effects, such as gastrointestinal and bone marrow toxicities, other than nephrotoxicity resulting from DDP administration in rats.

Experimental

Materials DDP was kindly provided by Nippon Kayaku Co., Ltd. (Tokyo). BGD was synthesized according to the procedure reported in our previous paper.[23] All other chemicals were of reagent grade.

Animal Male Wistar rats, weighing 200—220 g, were purchased from Kyudo Co., Ltd. (Kumamoto) and housed in individual metabolic cages with diet (Nosan Lab Chow) and drinking water *ad libitum*. The animals were maintained on a 12-h light/dark cycle and the temperature of the animal care facilities was 23 to 26 °C.

***In Situ* Excretion and Distribution Experiment** The rats were anesthetized with urethane (0.5 g/kg, i.p.) and the bile duct was cannulated with polyethylene tubing (PE 10) as described previously.[24] Thereafter, the rats were injected i.p. with saline or BGD immediately after i.v. injection of DDP (20 μmol/kg). In this and subsequent experiments, BGD was given at a dose of 2.0 mmol/kg, which was chosen as an effective dose for the prevention of DDP-induced renal toxicity.[22] Bile and urine samples were collected for an experimental period of 5 h. The rats were killed with urethane at the end of the experiment and the liver and kidney were removed for the determination of platinum (Pt).

Assay of Intestinal Disaccharidases Rats were injected i.p. with saline or BGD (2 mmol/kg) immediately after i.v. injection of saline or DDP (20 μmol/kg). After 4 d, the rats were killed with urethane. The disaccharidase activities of the intestinal mucosa were determined according to the method of Dahlquist.[25] Pieces of the jejunum were removed and chilled with ice. The jejunum was cut open and the mucosa was scraped off with a piece of glass and homogenized in 4 vol. of chilled 0.1 M maleate buffer (pH 6.0) with a glass-Teflon homogenizer. The homogenate was centrifuged at 4000 rpm for 10 min at 4 °C. An aliquot (0.1 ml) of the supernatant (enzyme solution) was mixed with 0.1 ml of the substrate solution (0.056 M maltose or sucrose) in the maleate buffer and incubated in a water bath at 37 °C. After 60 min the mixture was added to 0.8 ml of distilled water, heated in boiling water for 2 min to interrupt the enzyme reaction and then cooled with tap water. A blank with the same composition was heated in boiling water immediately after mixing of the enzyme and substrate. The amount of glucose present in the incubation mixture was determined by the method of Miwa *et al.*[26] using a commercially available kit, Glucose C-Test Wako (Wako Pure Chemical Ind., Osaka).

Histology The effect of BGD on the jejunal mucosa of DDP-treated rats was examined. The same protocol used in animal experiments was employed. The jejunum was cut into 5 × 5 mm strips. The strips were placed in 2% glutaraldehyde, washed in 0.1 M phosphate buffer (pH 7.4), and fixed in 1% osmium tetroxide for 2 h. The tissue was dehydrate through a gradual series of ethanol (25—100%) and isoamylacetate (25—100%) solutions, and dried directly from alcohol in a critical point dryer. The dried tissue was mounted on aluminum stubs, and a layer of gold-palladium was deposited with a sputtering unit. The tissue was examined with a Hitachi scanning electron microscope (model S-510).

Determination of Leukocytes The number of leukocytes was counted using a hemacytometer after dyeing with a Turk solution (Nakarai Kagaku Ind., Kyoto).

Analytical Procedures The bile, urine, liver, and kidney were wet-ashed by the $HClO_4$–HNO_3 method,[27] and Pt levels in these specimens were determined by a Hitachi Zeeman effect flameless atomic absorption spectrometer (model Z-8000). The calibration curve for Pt was prepared using standard solutions of 0.25—2.50 ppm.

Statistical Analysis Data were analyzed by a one-way analysis of variance. When the analysis indicated that a significant difference existed, the treated groups were compared to the controls by Duncan's new multiple range test.

Results and Discussion

The effects of DDP and/or BGD on the rat gastrointestinal tract were investigated. As shown in Fig. 1, a scanning electron micrograph of the jejunum 4 d after DDP treatment demonstrated mucosal damage and a clear reduction in the villus height, compared with the control, as reported in the paper of Allan *et al.*[28] The micrograph of the jejunum after DDP with BGD showed only a slight abnormal appearance, indicating the protective effect of BGD against DDP-induced damage in the villi.

The functional activity of enterocytes at 4 d after DDP injection was examined by disaccharidase activity in the jejunal portions. The effect of DDP on the activities of maltase and sucrase in the jejunal mucosa and the effect of BGD on these changes are shown in Table I. DDP caused a significant depletion in both maltase and sucrase activities. BGD coadministration significantly reversed the reduction in disaccharidase activity following DDP injection, but did not return the activity to the control value level. In addition, diarrhea was observed after DDP injection (Table I). BGD treatment had a protective effect against DDP-induced diarrhea.

In order to further investigate this gastrointestinal toxicity of DDP, we examined the Pt distribution in the gastrointestine as well as in the kidney and liver following injection of DDP and/or BGD in rats (Table II). Concentrations of Pt in the stomach and small intestine after DDP injection were much smaller than those in the kidney and liver. BGD treatment significantly decreased Pt concentrations in the gut, resulting in protective effect of BGD against gastrointestinal toxicity by DDP. Also, a remarked reduction in renal Pt concentration following BGD treatment resulted in the protection of DDP-induced renal damage, as reported in our previous paper.[22]

Recent studies[29,30] showed that diethyldithiocarbamate protected the bone marrow toxicity of DDP in mice. We also examined the effect of BGD on bone marrow toxicity induced by DDP. As shown in Table III, DDP alone caused a significant decrease in total leukocytes. However, the reduction of leukocytes produced by DDP returned to control values after BGD treatment, indicating the protective effect of BGD against DDP-induced bone mar-

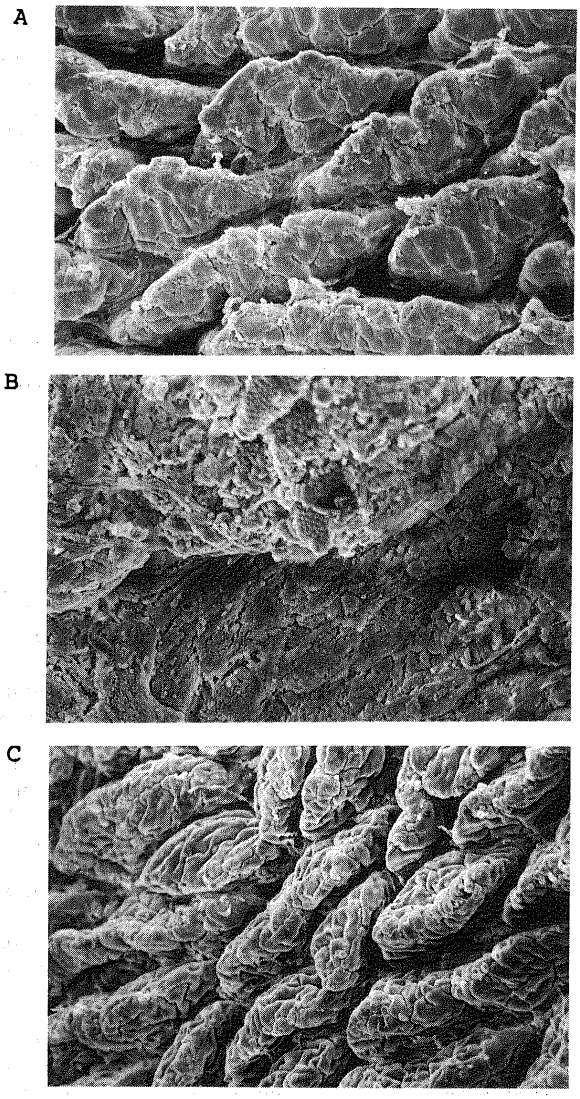

Fig. 1. Scanning Electron Micrograph of Rat Jejunum Following DDP Injection

A, control rat jejunum stained with 1% osmium tetroxide and coated with gold-palladium; B, rat jejunum 4 d after DDP (20 μmol/kg); C, rat jejunum 4 d after DDP (20 μmol/kg) and BGD (2 mmol/kg). Magnification, ×150.

Table I. Effect of BGD on Gastrointestinal Toxicity of DDP

Treatment	Enzyme activity[a] (μmol hydrolyzed/min/g mucosa)		Diarrhea observed
	Maltase	Sucrase	
Control	8.29±0.96	2.03±0.17	0/4
DDP alone	4.95±0.08[b]	1.13±0.05[b]	3/4
DDP+BGD	5.82±0.54[b,c]	1.37±0.07[b,c]	0/4

The rats were injected i.p. with saline or BGD (2 mmol/kg) immediately after i.v. injection of saline or DDP (20 μmol/kg). After 4 d, the rats were killed and the maltase and sucrase activities of the jejunum were determined. *a*) The values represent the mean±S.D. for 4 animals. *b*) Significantly different from control, $p<0.01$. *c*) Significantly different from DDP alone, $p<0.05$.

Table II. Effect of BGD of Tissue Concentration of Pt after DDP Injection

Tissue	Concentration of Pt (μg/g wet tissue)[a]	
	DDP alone	DDP+BGD
Stomach	0.49±0.03	0.15±0.05[b]
Small intestine	0.26±0.03	0.12±0.03[b]
Kidney	8.70±0.77	2.62±0.38[b]
Liver	2.49±0.23	0.61±0.06[b]

The rats were injected i.p. with saline or BGD (2 mmol/kg) immediately after i.v. injection of saline or DDP (20 μmol/kg). After 4 d, the rats were killed and the tissue concentration was determined by the atomic absorption method. *a*) The values represent the mean±S.D. for 4 animals. *b*) Significantly different from DDP alone, $p<0.01$.

Table III. Effect of BGD on Bone Marrow Toxicity of DDP

Treatment	Total leukocytes[a] (cells/ml)
Control	9820±1070
DDP alone	4910± 390[b]
DDP+BGD	8700± 520[c]

The rats were injected i.p. with saline or BGD (2 mmol/kg) immediately after i.v. injection of saline or DDP (20 μmol/kg). After 4 d, a blood sample was collected. *a*) The values represent the mean±S.D. for 4 animals. *b*) Significantly different from control, $p<0.01$. *c*) Significantly different from DDP alone, $p<0.01$.

TABLE IV. Effect of BGD on Excretion and Tissue Distribution of Pt after DDP Injection

Sample	Pt (% of dose)[a]	
	DDP alone	DDP+BGD
Bile	0.82±0.23	31.19±10.61[b]
Urine	4.88±2.26	16.44± 4.09[b]
Kidney	3.79±0.75	3.30± 1.47
Liver	4.98±0.58	17.34± 5.49[b]

The rats were injected i.p. with saline or BGD (2 mmol/kg) immediately after i.v. injection of DDP (20 μmol/kg), and urine samples were collected for 5 h. At the end of the experiment, the rats were killed and the tissue distribution of Pt was determined by the atomic absorption method. *a*) The values represent the mean±S.D. for 4 animals. *b*) Significantly different from DDP alone, $p<0.01$.

row toxicity.

Moreover, we investigated biliary and urinary excretion of Pt after injection of DDP and/or BGD in rats (Table IV). As is evident, there were marked increases in the Pt contents of the bile and urine subsequent to BGD administration. During the short time (5 h) during which the biliary and urinary excretion of Pt was measured, there was no significant change in the renal Pt content of rats treated with BGD compared with DDP alone. During the course of these experiments, the hepatic Pt content significantly increased compared with that of DDP alone. As shown in Table II, however, the Pt concentrations in the kidney and liver of rats injected with DDP significantly decreased compared with those of DDP alone 4 d after BGD treatment.

BGD is considered to be involved in the formation of complex that includes Pt binding at certain sites, such as the kidney, the gastrointestinal tract, and the bone marrow. Thus, it allows the removal of Pt from the sites without altering the antitumor response of DDP.[22]

In summary, the present study reveals that the injection of BGD to rats treated with DDP can effectively remove Pt from the body through biliary and urinary excretion, resulting in a protective effect of BGD against gastrointestinal and bone marrow toxicities produced by DDP injection.

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