

Effect of Platinum Coordination Complex (PtCx) on Citrate Uptake by Rat Renal Brush Border Membrane Vesicles (BBMV): Cisplatin-Intoxicated Rats

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Received March 1, 2001 and accepted July 10, 2001

Abstract: Platinosis with severe dermatitis and/or asthma is broadly defined as the effects of soluble platinum salts on people exposed to them occupationally. Platinum coordination complexes are widely used in the treatment of a variety of solid tumors. However, the clinical use of cisplatin, the most useful agent, is limited by the development of nephrotoxicity. Thus, an accidental exposure to soluble platinum at a high dose in platinum refineries and pharmaceutical factories could induce occupational nephrotoxicity. Urinary citrate is freely filtered at the glomerulus, and its reabsorption in the proximal tubule is the major determinant of the rate of renal excretion. In our previous studies, we found that the preincubation of rat renal brush border membrane vesicles (BBMV) with cisplatin and carboplatin, a second-generation platinum coordination complex, significantly inhibited the citrate uptake compared with that of the control BBMV. In this study, we performed *in vivo* experiments in cisplatin-intoxicated rats to elucidate the toxic mechanism of cisplatin. And our results showed that the citrate uptake was significantly inhibited in cisplatin-intoxicated rats at 1 min compared with that of control rats.

Key words: Platinum, Cisplatin, Citrate, Brush border membrane vesicles

Introduction

Roberts¹⁾ reported the occurrence of “platinosis” among workers in a laboratory using and refining platinum. Platinosis is considered to be a progressive allergic reaction following exposure to platinum salts, which leads to pronounced asthmatic symptoms. Platinum coordination complexes (PtCx) are potent against several types of cancer but are nephrotoxic²⁾. Cisplatin (cis-diamminedichloroplatinum II, CDDP) is one of the most useful chemotherapeutic agents, but its clinical use is limited by its acute nephrotoxic potential. CDDP nephrotoxicity is characterized by early impairment of water and solute tubular transport processes, leading to marked increases in urinary excretion of sodium, magnesium, and

glucose. CDDP inhibits the activity of several proteins with SH groups essential for the activity of glucose transporters of the rabbit renal brush border membrane³⁾. However, the underlying mechanisms have not been explored⁴⁾. Kim *et al.*⁴⁾ reported that cisplatin treatment, a single i.p. exposure of cisplatin, at a dose of 4 mg/kg did not affect the Na⁺-dependent uptake of glucose and L-glutamate by brush-border membrane vesicles (BBMV), but caused a significant decrease in Na⁺-dependent succinate and H⁺-dependent TEA uptake. Thus, high dose accidental exposure of soluble platinum at a platinum refinery or pharmaceutical factory could possibly induce occupational nephrotoxicity.

Renal citrate excretion is important in terms of both the prevention of kidney stones and acid-base balance. Citrate is freely filtered at the glomerulus, and its reabsorption in the proximal tubule is the major determinant of the rate of

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renal excretion⁵). In our previous studies^{6,7}, we reported that the preincubation of rat renal brush border membrane vesicles (BBMV) with 5 mM cisplatin for 4 and 8 hours and 100 mM carboplatin, a second-generation platinum coordination complex, for 8 hours significantly inhibited the citrate uptake compared with that of the control BBMV.

In this study, we performed *in vivo* experiments in cisplatin-intoxicated rats to elucidate the toxic mechanism of cisplatin and to confirm findings in *in vitro* experiments.

Materials and Methods

Materials

¹⁴C-citrate (spc. act. 1.85 GBq/m mol) was obtained from the New England Nuclear Corp. (Boston, MA). All other chemicals were reagent grades and purchased from commercial sources.

Animals

Experiments were performed using male Wistar rats (CLEA, Japan, Inc.) weighing 290–420 g. Animals were maintained in a temperature- and photoperiod-controlled animal house with *ad libitum* access to a standard diet and tap water. Cisplatin-intoxication was induced by a single intraperitoneal (i.p.) injection of cisplatin (5 mg/kg body wt).

Brush border membrane isolation

Each brush border membrane specimen was prepared from the cortex of both kidneys of a single rat. During preparation, all materials were maintained at 4°C. The brush border membrane specimens were isolated by differential centrifugation and magnesium precipitation technique⁸. Briefly, each rat was anesthetized with chloral hydrate (360 mg/kg body wt) administered intraperitoneally on the 6th day after the drug injection. The renal cortices were removed and placed in isolation buffer consisting of 50 mM mannitol and 2 mM Tris-(hydroxymethyl)-aminomethane (Tris)/HCl at pH 7.0. They were then homogenized in a glass Teflon homogenizer (Iuchi Co. Ltd., Japan) and a polytron homogenizer. A 1 M concentration of MgCl₂ was added to the resultant homogenate to give a final concentration of 10 mM. This mixture was stirred for 20 min in a cold room and then centrifuged for 15 min at 3,000 g. The supernatant was collected and centrifuged for 20 min at 43,000 g. The pellet containing the brush border membrane vesicles was resuspended in the isolation buffer and centrifuged for 15 min at 3,000 g. The supernatant was centrifuged for 20 min at 43,000 g. The pellet containing the purified brush border membrane vesicles (BBMV) was resuspended in 260 mM

mannitol and 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)/Tris at pH 7.0.

Marker enzyme assays

The purity of the membrane preparations was assessed by evaluation of the enrichment of alkaline phosphatase (Alp)⁹ and leucine aminopeptidase (LAP)¹⁰ in the final preparation as compared with that of the original homogenate.

Protein determination

The protein determination was conducted by the method of Lowry *et al.*¹¹ using crystalline bovine serum albumin as the standard.

Uptake studies

Since citrate uptake is sodium dependent and electrogenic, positive charge is transported¹², transport measurements were determined by the Millipore rapid membrane filtration technique^{13–15}. In a typical uptake experiment, BBMV (10 μ l) were incubated at 30°C with 40 μ l of uptake solution consisting of 100 μ M ¹⁴C-citrate, 130 mM NaCl or KCl and 20 mM HEPES/Tris, pH 7.0. At appropriate intervals, the uptake was terminated by the addition of 4 ml of ice-cold solution consisting of 130 mM NaCl or KCl and 20 mM HEPES/Tris at pH 7.0. The solution was then rapidly filtered through a Millipore filter (DAWP02500; pore size: 0.65 μ m) and washed twice with 4 ml of ice-cold stop solution under vacuum suction. The back ground uptake was determined similarly by adding 4 ml of ice-cold solution to 10 μ l membrane vesicles before adding 40 μ l of uptake solution at 4°C. The non-specific binding was always < 0.5% of the total counts in the uptake solution. Radioactivity associated with the filters was measured using a liquid scintillation counter (LSC-3500, Aloka, Japan). All uptake measurements were performed at 30°C in triplicate, and uptake was calculated on the basis of the specific activity measured in each experiment. The value of non-specific binding was subtracted from the experimental value, and the vesicular uptake was expressed as picomoles ¹⁴C-citrate per mg protein.

Statistical analysis

Values are shown as means \pm S.E. Student's t-test was used to analyze differences among the groups. Significance was accepted at $p < 0.05$.

Results

The purification of the BBMV was assessed by the BBM-bound specific enzymes, Alp and LAP. The enrichment of

Table 1. Blood analysis and serum electrolytes

	Control (n=5)	Cisplatin-treated (n=5)
pH	7.40 ± 0.01	7.32 ± 0.05
pCO ₂ , mmHg	44.0 ± 0.7	46.3 ± 1.7
HCO ₃ , mM	26.0 ± 0.7	22.5 ± 2.5
Na, mM	136 ± 0.5	133 ± 2.0
K, mM	3.8 ± 0.06	4.1 ± 0.2

Values are means ± S.E. *, p<0.05.

these enzymes in the BBMVs was about 10-fold higher than those in the original homogenate.

Body weight and plasma measurements

The body weights before i.p. injection were not significantly different between the two groups (330 ± 20 in the control, n=5 vs. 351 ± 19 in the cisplatin-treated rats, n=5). The i.p. injection of cisplatin induced weight loss (control: 361 ± 19 vs. cisplatin-treated: 298 ± 29) after 6 days, but there was not significant difference between the two groups. The arterial blood gases and plasma electrolytes were also not significantly different between the two groups (Table 1).

Time course of citrate uptake by the BBMVs in cisplatin-intoxicated rats

Since BBMVs were prepared in NaCl-free medium and incubated in 130 mM NaCl-containing buffer, the uptake of citrate showed a characteristic 'overshoot' phenomenon during the first 2 min and reached equilibrium after 120 min in the Na⁺-gradient groups (Fig. 1). The overshoot represents an intravesicular accumulation of citrate above the equilibrium concentration and occurs by the intravesicular citrate had already reached the concentration in the incubation medium¹⁶. Figure 1 shows the time course of citrate uptake by BBMVs of the control and cisplatin-treated rats. Values of transport at 1 min were significantly different between the Na⁺-gradient two groups (p<0.05). The overshoot phenomenon was less marked in cisplatin-intoxicated rats. When the Na⁺-gradient was replaced by a K⁺-gradient, the initial uptake of citrate was markedly low and the overshoot did not occur (Fig. 1).

Discussion

Cisplatin is one of the most useful currently available chemotherapeutic agents. Although renal toxicity has been reduced somewhat by the routine use of hydration and

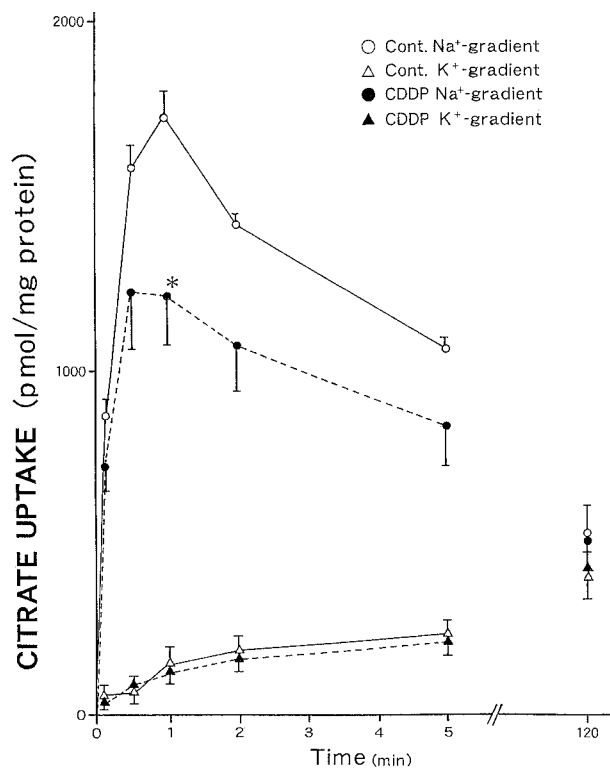


Fig. 1 Time course of Na⁺-dependent and independent citrate uptake by rat renal brush border membrane vesicles (BBMV) in control and cisplatin-intoxicated rats.

Uptake solution consisting of 100 μM ¹⁴C-Citrate, 130 mM NaCl () or KCl () and 20 mM HEPES/Tris at pH 7.0 was added to vesicles consisting of 260 mM mannitol and 20 mM HEPES/Tris at pH 7.0. Values are means ± S.E. *P<0.05 vs. control.

mannitol diuresis, it remains a major problem¹⁷, and carboplatin (cis-diammine-1,1-cyclobutane-dicarboxylate platinum (II), CBDCA, a second-generation platinum coordination complex) is less nephrotoxic than CDDP at therapeutic doses¹⁸. Our previous results^{6,7} showed that the preincubation of BBMVs with 100 mM carboplatin for 8 hours also significantly inhibited the citrate uptake compared with that of the control BBMVs, but the alterations were not as severe as those with 5 mM cisplatin.

Proximal tubular absorption is increased in metabolic acidosis and inhibited in metabolic alkalosis^{12,19}. It is considered that the metabolic acidosis was probably caused by diminished excretion of ammonium or retention of acidic metabolites due to the marked decrease in GFR²⁰. However, metabolic acidosis was not noted in the present study (Table 1). Lacchini *et al.*²⁰ reported that cortical distal tubule acidification, measured by double-barreled ion-exchange resin/PD microelectrodes, was not significantly affected by cisplatin. This accounted for the observation that, in spite

of the impaired proximal acidification, urine pH was kept within the normal range²⁰. Cortical distal acidification might not be impaired and metabolic acidosis was not observed in this study. Thus, we speculated that we may have observed the early phase of cisplatin-induced nephrotoxicity in which cortical distal acidification was not impaired in spite of impaired proximal acidification.

CDDP induced a concentration-dependent inhibition of Na⁺-coupled uptake of α -methylglucopyranoside (MGP), phosphate (Pi) and alanine in BBM vesicles from the renal cortex, associated with a decrease in protein sulfhydryl content. The CDDP-induced inhibition of Na⁺/Pi and Na⁺/glucose cotransport systems may be mainly mediated by direct chemical interactions with essential sulfhydryl groups in the transporters²¹. In this study, the values of transport at 1 min were significantly different between the Na⁺-gradient two groups ($p < 0.05$, Fig. 1). The direct inhibitory effect of cDDP on protein sulfhydryl content in renal BBM vesicles could be due to the strong chemical reactivity of cDDP with biological nucleophiles²¹. Sugihara K *et al.*²² indicate that the production of free radicals, due to the formation of lipid peroxides that damage membrane functions, may be responsible for nephrotoxicity. We speculated that the down-regulation of transporters might occur in this system, which might contribute to nephrotoxicity in cisplatin therapy.

Several studies have suggested that CBDCA is less reactive than CDDP with biological nucleophiles such as the SH group of cysteine residues due to the different kinetics of the aquation reactions of the two compounds²³. In chloride-free medium, the hydrolysis of CDDP chloride ligands, which leads to the formation of hydrated species that are highly reactive with biological nucleophiles, occurred 100-times more rapidly than the hydrolysis of cBDCA carboxylate ligands²³. In conclusion, more effective and less nephrotoxic platinum coordination complexes at therapeutic doses are needed.

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