

Effect of Platinum Coordination Complex (PtCx) on Citrate Uptake by Rat Renal Brush Border Membrane Vesicles (BBMV): Direct Effect of Carboplatin

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Abstract: Inhalation of platinum, as soluble salts, is known to cause respiratory distress and severe dermatitis in workers. Platinum coordination complexes are widely used in the treatment of a variety of solid tumors. However, the clinical use of cisplatin (CDDP) (the most useful agent) is limited by the development of nephrotoxicity. High dose accidental exposure to soluble platinum in platinum refineries and pharmaceutical factories could induce occupational nephrotoxicity. Carboplatin (CBDCA), a second-generation platinum coordination complex, is highly effective against a variety of malignancies at doses five- to ten-times higher than CDDP. At therapeutic doses, CBDCA is less nephrotoxic than CDDP. Additionally, 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 study, the preincubation of rat renal brush border membrane vesicles (BBMV) with 5 mM cisplatin for 4 and 8 hours significantly inhibited the citrate uptake compared with that of the control BBMV. In this study, we exposed BBMV to 100 mM carboplatin (twenty-times higher concentration than cisplatin) and examined the citrate uptake characteristics to clarify the toxic mechanism of platinum coordination complexes. The preincubation of BBMV with 100 mM carboplatin for 8 hours also significantly inhibited the citrate uptake compared with that of the control BBMV, but the alterations were not as severe as those with 5 mM cisplatin.

Key words: Carboplatin (CBDCA), Nephrotoxicity, Renal brush border membrane vesicles (BBMV), Citrate

Campbell *et al.*¹⁾ investigated the dermal irritancy of platinum compounds in albino rabbits. Platinum tetrachloride was rated as unsafe for intact and/or abraded skin contact, while platinum dichloride and platinum dioxide were considered only a minor hazard for intact or abraded skin contact. Levene²⁾ noted that the allergic hypersensitivity to platinum salts resulted from induced histamine release elicited by worker exposure to hexachloroplatinate intermediates in platinum refining operations. Cisplatin [cis-diamminedichloroplatinum (II), CDDP] is a very potent drug effective against a variety of solid tumors, but its clinical

use is limited by its acute nephrotoxic potential³⁾. Carboplatin [cis-diammine-1,1-cyclobutane-dicarboxylate platinum (II), CBDCA] is less nephrotoxic than CDDP at therapeutic doses⁴⁾.

Renal citrate excretion is important with regard to 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 renal excretion⁵⁾. In our previous study⁶⁾, the preincubation of rat renal brush border membrane vesicles (BBMV) with 5 mM cisplatin for 4 and 8 hours significantly inhibited the citrate uptake by BBMV compared with control rats, no preincubation of cisplatin. In this study, to clarify the toxic

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mechanism of differences in platinum coordination complexes, we exposed BBMVs to 100 mM carboplatin and measured the citrate uptake by BBMVs.

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

The animals used in these experiments were SPF male Wistar rats (CLEA, Japan, Inc.) weighing 330–359 g ($n=5$). They were maintained in a temperature- and photoperiod-controlled animal house with *ad libitum* access to a standard diet and tap water.

Each membrane specimen was prepared from the cortex of both kidneys of a single rat by a modification of the MgCl_2 -precipitation technique⁷ and used within 10 h after preparation. During preparation, all materials were maintained on ice or at 4°C. Briefly, the rats were anesthetized with an injection of chloral hydrate (360 mg/kg body wt) intraperitoneally. The renal cortices were removed and placed in an isolation buffer consisting of 50 mM mannitol and 2 mM tris-(hydroxymethyl)-aminomethane (Tris)/HCl (pH 7.0). They were homogenized in a glass Teflon homogenizer (Iuchi Co. Ltd., Japan) and a polytron homogenizer. A concentration of MgCl_2 (1M) 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 BBMVs was resuspended in isolation buffer and centrifuged for 15 min at 3,000 g. The supernatant was collected and centrifuged at 43,000 g. The final pellet containing the purified BBMVs was resuspended in 260 mM mannitol and 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)/Tris (pH 7.0).

The purity of membrane preparations was assessed from the enrichment of alkaline phosphatase (Alp)⁸ and leucine aminopeptidase (LAP)⁹ in the final preparation as compared with that of the original homogenate.

Protein determination was conducted by the method of Lowry *et al.*¹⁰ with crystalline bovine serum albumin as the standard. Transport measurements were performed in freshly isolated BBMVs by the Millipore rapid membrane filtration technique^{11,12}. For the experiment in which BBMVs were preincubated with carboplatin, membrane vesicles (10 μl) were preincubated at 30°C with 20 μl of preincubation solution consisting of 260 mM mannitol and 20 mM HEPES/Tris (pH 7.0) with or without 100 mM carboplatin for an appropriate interval. Citrate uptake was initiated by the addition of 40 μl of uptake solution consisting of 100 μM

^{14}C -citrate, 130 mM NaCl and 20 mM HEPES/Tris (pH 7.0). After thirty seconds, the uptake was terminated by the addition of 4 ml of ice-cold solution consisting of 130 mM NaCl and 20 mM HEPES/Tris (pH 7.0). The solution was then rapidly filtered through a Millipore filter (DAWP02500; pore size 0.65 μm) and washed twice with 4 ml of ice-cold stop solution under vacuum suction. The background uptake was similarly determined by adding 4 ml of ice-cold stop solution to membrane vesicles (10 μl) before adding 40 μl of uptake solution at 4°C. The non-specific binding was always <0.5% of the total count in the uptake solution. The radioactivity associated with the filters was measured with a liquid scintillation counter (LSC-3500, Aloka). 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 non-specific binding value was subtracted from the experimental value. The vesicular uptake is expressed as picomoles ^{14}C -citrate per mg protein and presented as means \pm S.E. Student's t-test was used to analyze differences among groups. Significance was accepted at $P<0.05$.

The purity of the BBMV preparations was assayed by the determining the BBM-specific marker enzymes Alp and LAP. The specific activities of the two enzymes in the BBM fraction were enriched to a 10-fold higher level than those in the original homogenate.

A 4 h preincubation with 100 mM carboplatin inhibited the citrate uptake compared to that without carboplatin, but not significantly. A 8 h preincubation significantly inhibited the citrate uptake compared to that without carboplatin, and induced an approx. 60% reduction (Fig. 1). However, its alterations were not as severe as those with 5 mM CDDP⁶.

These findings indicated that CBDCA altered the citrate uptake at concentrations more than 20-times higher than those of CDDP. Potdevin *et al.*¹³ reported that CBDCA concentrations approximately thirty-times greater than those of CDDP were required to obtain a similar extent of inhibition of Na^+ -dependent glucose uptake, similar decreases in protein SH content and similar platinum binding to BBM vesicles. A similar order of concentration difference has been observed regarding inhibition of Na^+ -dependent glucose uptake in proximal tubular cells in primary culture¹⁴. Our results are consistent with these data.

Several studies suggest that CBDCA is less reactive than CDDP with biological nucleophiles such as the SH group of cysteine residues owing to the different kinetics of the aquation reactions of the two compounds¹⁵. In chloride-free medium, hydrolysis of CDDP chloride ligands, which leads to the formation of hydrated species that are highly

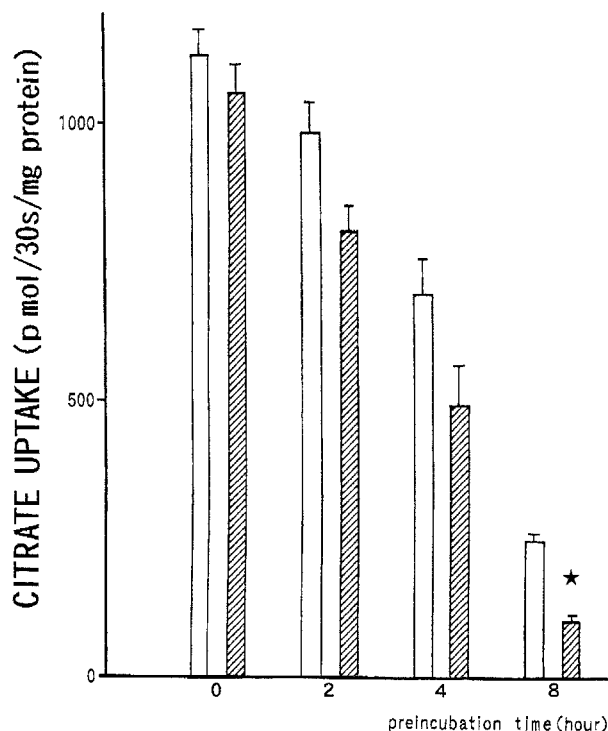


Fig. 1. Effect of carboplatin on citrate uptake by rat renal brush border membrane vesicles (BBMV) with respect to pre-incubation time (0-8 h, n=5).

Values are means \pm S.E. \square : preincubation with 0 mM carboplatin (control), ▨ : preincubation with 100 mM carboplatin. $P < 0.002$ vs control.

reactive with biological nucleophiles, is 100-times more rapid than the hydrolysis of CBDCA carboxylate ligands¹⁵).

Our *in vitro* studies showed that cisplatin is highly nephrotoxic. To clarify further the toxic mechanism of platinum coordination complexes and to confirm these *in vitro* experiments, *in vivo* experiments in cisplatin-intoxicated rats are needed more.

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