Cancer Chemother Pharmacol (1985) 15: 93–96

Cancer Chemotherapy and Pharmacology

Original articles

The effect of cisplatin on renal ATPase activity in vivo and in vitro

Jiro Uozumi and Charles L. Litterst

Laboratory of Pharmacology and Experimental Therapeutics, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute,
National Institutes of Health, Bethesda, MD 20205, USA

Summary. The effect of cisplatin on ATPase activity was determined in vitro and in vivo to investigate the correlation between nephrotoxicity and the inhibition of ATPase activity by cisplatin. Purified Na,K-ATPase was preincubated for 0–240 min with cisplatin at concentrations of 50–800 μ*M* in vitro before the determination of enzyme activity. Although ATPase activity was reduced by cisplatin, either a high concentration of cisplatin (280 μ*M*) or a long period of preincubation (160 min) with cisplatin was required to obtain 50% inhibition of ATPase activity. Similar in vitro experiments using kidney homogenate from female Sprague-Dawley rats instead of purified Na,K-ATPase were performed. Activity of Na,K-ATPase in rat kidney homogenate was inhibited by 50% after 110 min preincubation with 800 μ*M* cisplatin or 160 min preincubation with 400 μ*M* cisplatin. Female Sprague-Dawley rats were given 5, 7 or 10 mg/kg of cisplatin IV and BUN level, ATPase activity and Pt concentration in kidney homogenate were evaluated 1 h, 6 h, 1 day, 3 days, and 5 days after cisplatin injection. In rats given 10 mg/kg cisplatin a significant increase of BUN was observed on days 1, 3, and 5. In rats treated with 5 or 7 mg/kg of cisplatin BUN was increased on days 3 and 5. Normal ATPase activity, however, was preserved until day 3 at all doses. The highest concentration of Pt observed in kidney tissue was 19.3 μg/g tissue. This value was insufficient to inhibit ATPase activity significantly in vitro. Thus, it seems unlikely that the inhibition of ATPase activity is the cause of nephrotoxicity, although cisplatin can affect ATPase activity.

Introduction

Cisplatin is known to be a nephrotoxic anticancer agent [7, 13]. In clinical use, its nephrotoxicity is one of the most important dose-limiting factors [17]. However, the mechanism of cisplatin-induced nephrotoxicity has not been well determined. Guarino et al. [9] suggested that the biochemical site of cisplatin toxicity is on ATPase, basing this on their in vitro studies performed with isolated flounder tubules and rat kidney homogenates. Daley-Yates and McBrien [6] reported that ATPase activity in rat kidney homogenate was inhibited by in vitro incubation with cisplatin. Most recently, Nechay and Neldon [15] found that ATPase activity in human kidney homogenate was inhibited by in vitro incubation with cisplatin. However, no one has yet investigated the effect of cisplatin injection on ATPase activity in vivo. The present study describes the inhibitory effect of cisplatin on ATPase activity not only in vitro but also after in vivo administration to rats.

Offprint requests to: C. L. Litterst

Materials and methods

cis-Diamminedichloroplatinum (II) (cisplatin) was obtained from the Drug Development Branch, NCI. Purified ATPase from dog kidney, ATP, and ouabain were purchased from Sigma Chemical Co. (St. Louis, Mo). Female Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 190 ± 20 g were used; they had free access to food and water.

In the ATPase assay, buffer containing 50 m*M* Tris, 100 m*M* NaCl, 20 m*M* KCl, 5 m*M* $MgCl_2$, 0.5 m*M* $CaCl_2$, and 1 m*M* EDTA, pH 7.4 was used. The enzyme activity was estimated by the release of inorganic phosphate from ATP.

In the in vitro experiments, purified ATPase (0.045 units; 1 unit = 1 μmol Pi/min) and cisplatin (0–800 μ*M*) were preincubated in 0.5 ml buffer for 0–240 min at 37 °C. ATP solution (0.5 ml) with or without ouabain was then added and the mixture incubated for a further 15 min at 37 °C to determine enzyme activity. The final concentrations of ATP and ouabain were 3 m*M* and 1 m*M*, respectively. After incubation, 1.0 ml 10% ice-cold trichloroacetic acid (TCA) was added and the mixture centrifuged for 15 min at 1000 *g*. Inorganic phosphate in the supernatant was measured by the method of Fiske and Subbarow [8]. The activity of Na,K-ATPase was determined by the difference between total ATPase activity measured in the absence of ouabain, and Mg-ATPase activity measured in the presence of ouabain. In addition to purified Na,K-ATPase, ATPase from rat kidney homogenate was also studied in vitro. Rat kidney was homogenized with a Potter-Elvejhem homogenizer in 10 vol. ice-cold incubation buffer. The homogenate was centrifuged at 600 *g* for 5 min to remove red blood cells and other debris. The supernatant was diluted 1:10 with buffer, and a 0.1 ml aliquot was mixed with 0.4 ml of buffer containing cisplatin. The mixture was preincubated for various times before the assay for ATPase activity. The concentration of cisplatin in the preincubation mixture, the preincubation time, and the procedures after preincubation were the same as those described above.

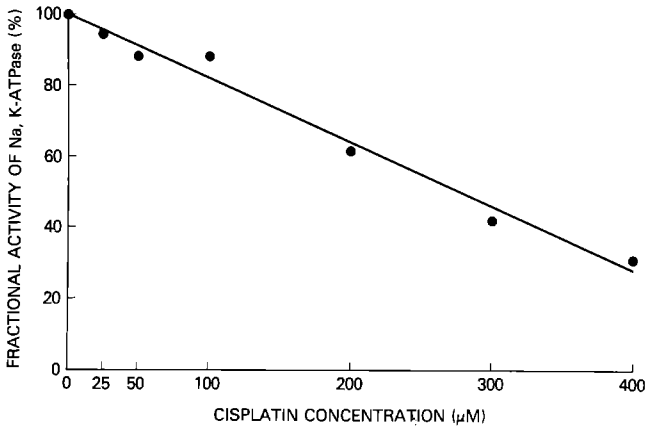

Fig. 1. Activity of purified Na,K-ATPase determined in the presence of cisplatin in vitro. Purified Na,K-ATPase was assayed in the presence of various concentrations of cisplatin, but with no preincubation. See *Methods* for assay conditions. Each *point* represents the average value for 3–6 samples

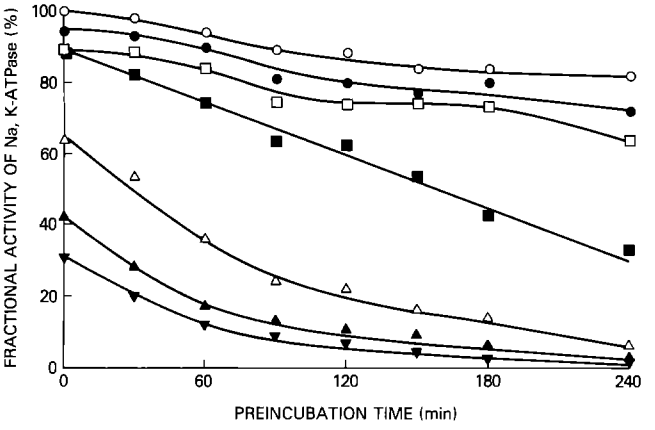

Fig. 2. Activity of purified Na,K-ATPase preincubated with cisplatin in vitro. Purified Na,K-ATPase was preincubated with 0 μ*M* (○), 50 μ*M* (●), 100 μ*M* (□), 200 μ*M* (■), 400 μ*M* (△), 600 μ*M* (▲), or 800 μ*M* (▼) cisplatin for the times indicated at 37 °C before determination of enzyme activity. Each *point* represents the average value for 3–6 samples

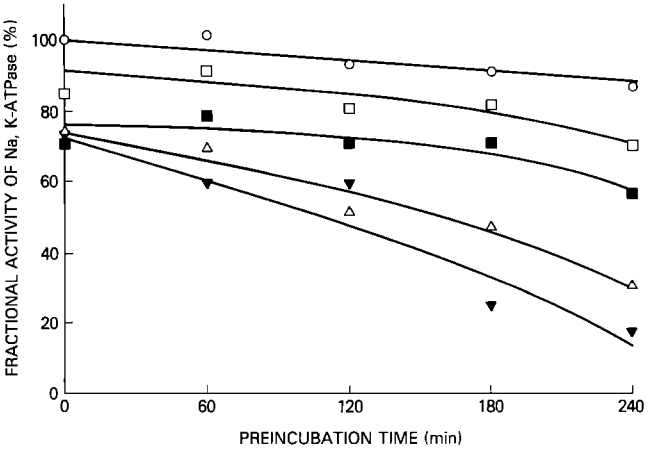

Fig. 3. Activity of rat kidney Na,K-ATPase preincubated with cisplatin in vitro. Rat kidney homogenate was preincubated with 0 μ*M* (○), 100 μ*M* (□), 200 μ*M* (■), 400 μ*M* (△), or 800 μ*M* (▼) cisplatin for the times indicates at 37 °C before determination of enzyme activity. Each *point* represents the average value for 3–6 rat samples

In the in vivo experiments, cisplatin dissolved in 0.9% NaCl was administered IV at doses of 5, 7, or 10 mg/kg. Animals were killed for analysis 1 h, 6 h, 1 day, 3 days, and 5 days after injection. Blood samples were taken for determination of urea nitrogen (BUN). For the assay of ATPase activity and Pt concentration, kidneys were removed and homogenized in the same manner as described above. A 0.1-ml aliquot was incubated with 0.9 ml ATP solution for 15 min at 37 °C in the absence or presence of ouabain to determine ATPase activity. Final concentrations of ATP and ouabain in the incubation mixture and the procedures after incubation were the same as in the in vitro experiments.

Pt concentration in kidney homogenate was measured by flameless atomic absorption spectrometry [11] after dilution with 0.25% Triton X-100. The recovery rate of Pt with this method was 99.3% ± 1.0%. BUN was assessed by the diacetyl monoxime method [5]. ATPase activity was indicated as the fractional activity relative to controls. Results were expressed as mean ± SD. Statistical analysis was performed by Student's *t*-test.

Results

Figure 1 shows the activities of purified Na,K-ATPase incubated with cisplatin for 15 min in vitro. The inhibition of enzyme activity became more severe with increasing cisplatin concentration. Thus, cisplatin concentrations of 100, 200, 300, and 400 μ*M* produced 12%, 38%, 58%, and 69% inhibition, respectively, of Na,K-ATPase activity. The concentration of cisplatin required for 50% inhibition of ATPase activity (I_{50}) was estimated to be 280 μ*M*.

Figure 2 indicates the activity of purified Na,K-ATPase decreased by 18% during the 240-min preincubation, even in the absence of cisplatin. A further decrease in enzyme activity according to preincubation time was found at all cisplatin concentrations. However, the decrease in activity was gradual when cisplatin concentration was less than 100 μ*M* and paralleled the line with no cisplatin. Thus, 36% of the activity was inhibited after preincubation in the presence of 100 μ*M* cisplatin for 240 min. Preincubation with cisplatin at concentrations of 200 μ*M* or 400 μ*M* reduced the Na,K-ATPase activity to 50% or below after 160 min or 30 min, respectively. When the cisplatin concentration was 600 μ*M* or 800 μ*M* preincubation resulted in 90% inhibition of the activity within 70 min or 110 min, respectively.

Figure 3 shows the activities of Na,K-ATPase in rat kidney homogenate incubated with cisplatin in vitro. Although cisplatin inhibited the Na,K-ATPase in rat kidney homogenate the inhibitory effect was less pronounced than with purified Na,K-ATPase. To obtain 50% inhibition of Na,K-ATPase activity, 160 min preincubation with 400 μ*M* cisplatin or 110 min preincubation with 800 μ*M* cisplatin was required.

Table 1 indicates the total ATPase, Mg-ATPase, and Na,K-ATPase activities in the kidney of rats treated with 5, 7, or 10 mg/kg cisplatin in vivo. There is no statistical difference, in either total ATPase or Mg-ATPase activity, between treated groups and controls until day 5. On day 5, total ATPase activities were 84%, 73%, and 62% in rats treated with 5, 7, and 10 mg/kg cisplatin, respectively. These values were all significantly lower than those in control animals, and a dose-related response is apparent. Mg-

Table 1. ATPase activities in kidney homogenate of rats treated with cisplatin in vivo

Time	Cisplatin dose (mg/kg)	% of total control activity		
		Total ATPase	Mg-ATPase	Na,K-ATPase
	Control	100.0 ± 7.4	85.9 ± 9.5	14.2 ± 4.1
1 h	5	99.2 ± 2.6	86.8 ± 5.9	12.4 ± 3.3
	7	99.7 ± 3.5	88.4 ± 4.2	11.3 ± 0.8
	10	99.9 ± 8.8	87.3 ± 5.0	12.6 ± 3.9
6 h	5	100.5 ± 11.4	89.2 ± 10.0	11.3 ± 1.6
	7	98.1 ± 1.7	85.8 ± 3.7	12.2 ± 2.4
	10	96.0 ± 1.6	85.1 ± 4.0	10.8 ± 4.0
1 day	5	96.2 ± 6.1	85.4 ± 5.9	10.8 ± 2.3
	7	103.8 ± 6.0	91.4 ± 3.4	12.4 ± 3.2
	10	99.5 ± 5.3	86.2 ± 4.0	13.4 ± 3.0
3 days	5	91.1 ± 5.2	75.1 ± 3.1	16.0 ± 2.3
	7	97.6 ± 8.6	81.3 ± 8.1	16.3 ± 0.7
	10	91.7 ± 5.1	76.1 ± 7.5	15.7 ± 2.4
5 days	5	84.3 ± 2.0*	75.8 ± 2.6	8.6 ± 0.6
	7	73.4 ± 12.2**	62.7 ± 8.8**	10.7 ± 4.4
	10	62.4 ± 10.1**	52.9 ± 6.3**	9.5 ± 3.9

Each value represents mean ± SD of 3–6 rats. Asterisks indicate statistically significant differences from controls: * $p \leq 0.01$; ** $p \leq 0.001$

Table 2. BUN levels (mg/100 ml) in rats after IV injection of cisplatin

Time	Cisplatin dose		
	5 mg/kg	7 mg/kg	10 mg/kg
1 h	17.1 ± 2.2	17.8 ± 1.2	16.2 ± 1.8
6 h	14.1 ± 2.3	13.5 ± 1.2	14.5 ± 2.1
1 day	20.2 ± 1.6	22.2 ± 1.6	23.5 ± 3.0*
3 days	34.3 ± 9.9**	42.5 ± 13.3**	67.7 ± 24.2**
5 days	114.7 ± 60.4**	124.1 ± 49.6**	161.9 ± 66.8**

Each value represents mean ± SD of 3–6 rats. Asterisks indicate statistically significant differences from controls (19.6 ± 2.8): * $p \leq 0.01$; ** $p \leq 0.001$

Table 3. Pt concentrations (μg Pt/g wet weight) in kidneys of rats following IV cisplatin

Time	Cisplatin dose		
	5 mg/kg	7 mg/kg	10 mg/kg
1 h	10.1 ± 1.1	15.2 ± 4.2	19.3 ± 3.7
6 h	8.9 ± 1.6	10.7 ± 0.4	14.0 ± 1.9
1 day	9.3 ± 1.5	13.3 ± 2.1	19.1 ± 2.6
3 days	10.7 ± 2.8	14.5 ± 3.2	16.9 ± 2.3
5 days	10.0 ± 0.9	11.2 ± 1.2	14.3 ± 1.0

Each value represents mean ± SD of 3–6 rats. There are no statistically significant differences in Pt concentrations between 1 h and any other time for any dose.

ATPase activity was also significantly lower in animals treated with 7 or 10 mg/kg cisplatin than in controls on day 5. However, there were no significant differences in Na,K-ATPase activities between any treatment group and controls at any time.

BUN was assessed to indicate renal function in rats employed for the in vivo ATPase assay (Table 2). Significant increases in BUN were found by day 3 in rats given 5 or 7 mg/kg cisplatin and by day 1 in rats that received 10 mg/kg cisplatin.

Table 3 indicates the Pt concentrations in kidney tissue of rats treated with cisplatin at doses of 5, 7 or 10 mg/kg. Kidney Pt concentrations were remarkably stable throughout the entire 5 days, with only a 25% decrease in concentration apparent at the two highest doses. However, there was a dose-related increase in tissue concentration of Pt, which was particularly obvious at the earlier times.

Discussion

Inhibition of ATPase activity by cisplatin is considered to be one of the possible mechanism of cisplatin-induced nephrotoxicity [3, 6, 9, 15]. Actually, several investigators have observed the inhibitory effect of cisplatin on ATPase in vitro [6, 9, 15]. However, in all these studies, high concentrations of cisplatin and/or long incubation times of ATPase with cisplatin were required for ATPase inhibition. For example, Guarino et al. [9] estimated that the concentration of cisplatin required for 50% inhibition of ATPase activity (I_{50}) was 5.0 m*M* for isolated flounder tubules and 2.0 m*M* for rat kidney homogenate. Daley-Yates and McBrien [6] obtained the value of 650 μ*M* for I_{50} of total ATPase when rat kidney homogenate was preincubated with cisplatin for 90 min. Nechay and Neldon [15] estimated that the I_{50} dose was approximately 700 μ*M* for 30-min preincubation in human kidney homogenate. Our present in vitro study demonstrates that concentrations of cisplatin lower than 100 μ*M* were unable to inhibit more than 50% of the activity of purified ATPase, even with a 240-min preincubation. In addition, the response of ATPase from rat kidney homogenate to cisplatin was qualitatively the same as that of purified ATPase.

The highest concentration of Pt in kidney tissue of rats treated with cisplatin in vivo was 19.3 μg Pt/g tissue, and this value is approximately equivalent to a molar concen-

tration of 96.7 μM Pt in the kidney. Thus, the maximum whole-kidney concentration barely achieves 100 μM, a concentration shown in vitro to produce less than 50% enzyme inhibition even after 4 h of preincubation. It might be assumed, then, that ATPase is not a primary site of action of cisplatin, because tissue concentrations never become high enough to cause a significant inhibition of enzyme activity. This conclusion assumes there is no selective accumulation of Pt in subcellular compartments that are rich in ATPase. Previous work has shown that the highest ATPase activities are observed mainly in mitochondria and plasma membrane [2, 16]. However, according to studies on subcellular distribution of Pt after cisplatin injection [4, 12, 18, 19], the highest concentrations of Pt in kidney tissue are localized in cytosol; relatively low concentrations were detected in mitochondria or plasma membrane fraction. The specific activity of Pt per milligram of protein in the mitochondria or plasma membrane fraction was lower than that in nuclei, microsome, or cytosol fraction. Thus, selective attack of cisplatin against ATPase in mitochondria or plasma membrane due to uncharacteristically high Pt concentrations is unlikely.

In our present study, ATPase activity in kidneys of cisplatin-treated rats was measured at relative short times (1 h and 6 h after cisplatin injection) as well as a longer times (1–5 days) after treatment. The kidney is exposed to unbound cisplatin, usually considered to be the active form of the drug, at high concentrations for a short interval after cisplatin injection [1, 10, 14, 20]. However, there was no significant decrease in ATPase activity either at 1 h or at 6 h after cisplatin injection (Table 1). This result seems to preclude the possibility of a temporary inhibition of ATPase that might cause significant impairment of renal function.

BUN is a widely recognized indicator of renal damage and was increased as early as day 1 after treatment (Table 2). However, there was no decrease in ATPase activity until day 5 after treatment. This tends to suggest that a decrease in ATPase activity is not an early event in cisplatin nephrotoxicity, but is more likely to be secondary to generalized tissue destruction in the kidney.

It is the conclusion of our present in vitro and in vivo experiment that the inhibition of ATPase activity by cisplatin seems to be unlikely as a cause of nephrotoxicity, although cisplatin can decrease ATPase activity in vitro.

References

1. Belt RJ, Himmelstein KJ, Patton TF, Bannister SJ, Sternson LA, Repta AJ (1979) Pharmacokinetics of non-protein-bound platinum species following administration of *cis*-dichlorodiammineplatinum (II). Cancer Treat Rep 63: 1515
2. Berg GG, Chapman B (1965) The sodium and potassium activated ATPase of intestinal epithelium. I: Location of enzymatic activity in the cell. J Cell Comp Physiol 65: 361
3. Borch RF, Katz JC, Lieder PH, Pleasants ME (1980) Effect of diethyldithiocarbamate rescue on tumor response to *cis*-platinum in a rat model. Proc Natl Acad Sci USA 77: 5441
4. Choie DD, Del Campo AA, Guarino AM (1980) Subcellular localization of *cis*-dichlorodiammineplatinum (II) in rat kidney and liver. Toxicol Appl Pharmacol 55: 245
5. Crocker CL (1967) Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am J Med Technol 33: 361
6. Daley-Yates PT, McBrien DCH (1982) The inhibition of renal ATPase by *cis*-platin and some biotransformation products. Chem Biol Interact 40: 325
7. Dentino M, Luft FC, Yum MN, Williams SD, Einhorn LH (1978) Long-term effect of *cis*-diamminedichloroplatinum (CDDP) on renal function and structure in man. Cancer 41: 1274
8. Fiske CF, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66: 375
9. Guarino AM, Miller DS, Arnold ST, Pritchard JB, Davis RD, Urbanek MA, Miller TJ, Litterst CL (1979) Platinate toxicity: past, present, and prospects. Cancer Treat Rep 63: 1475
10. Himmelstein KJ, Patton TF, Belt RJ, Taylor S, Repta AJ, Sternson LA (1981) Clinical kinetics of intact *cis*-platin and some related species. Clin Pharmacol Ther 29: 658
11. LeRoy AF, Wehling ML, Sponseller AL, Friauf WS, Solomon RE, Dedrick RL, Litterst CL, Gram TE, Guarino AM, Becker DA (1977) Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18: 184
12. Litterst CL, Schweitzer VG (1984) Increased tissue deposition and decreased excretion of platinum following administration of *cis*-platin to *cis*-platin-pretreated animals. Cancer Chemother Pharmacol 12: 46
13. Madias NE, Harrington JT (1978) Platinum nephrotoxicity. Am J Med 65: 307
14. Manaka RC, Wolf W (1978) Distribution of *cis*-platin in blood. Chem Biol Interact 22: 353
15. Nechay BR, Neldon SL (1984) Characteristics of inhibition of human renal adenosine triphosphatases by *cis*-platin and chloroplatinic acid. Cancer Treat Rep 68: 1135
16. Quigley JP, Gotterer GS (1969) Distribution of $(Na^+\text{-}K^+)$-stimulated ATPase activity in rat intestinal mucosa. Biochim Biophys Acta 173: 456
17. Schaeppi U, Heyman IA, Fleischman RW, Rosenkrantz H, Ilievski V, Phelan RA, Cooney DA, Davis RD (1973) *cis*-Dichlorodiammineplatinum (II) (NSC-119875): preclinical toxicologic evaluation of intravenous injection in dogs, monkeys and mice. Toxicol Appl Pharmacol 25: 230
18. Sharma RP, Edwards IR (1983) *cis*-Platinum: subcellular distribution and binding to cytosolic ligands. Biochem Pharmacol 32: 2665
19. Taylor DM, Jones JD, Robins AB (1973) Metabolism of platinum [^{14}C]ethylenediaminedichloride in the rat. Biochem Pharmacol 22: 833
20. Uozumi J, Ishizawa M, Iwamoto Y, Baba T (1984) Sodium thiosulfate inhibits *cis*-diamminedichloroplatinum (II) activity. Cancer Chemother Pharmacol 13: 82

Received December 18, 1984/Accepted February 8, 1985