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Cisplatin-induced Alterations of Na^+-dependent Phosphate Uptake in Renal Epithelial Cells

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Cisplatin treatment increases the excretion of inorganic phosphate *in vivo*. However, the mechanism by which cisplatin reduces phosphate uptake through renal proximal tubular cells has not yet been elucidated. We examined the effect of cisplatin on Na^+-dependent phosphate uptake in opossum kidney (OK) cells, an established proximal tubular cell line. Cells were exposed to cisplatin for an appropriate time period and phosphate uptake was measured using [^{32}P]-phosphate. Changes in the number of phosphate transporter in membranes were evaluated by kinetic analysis, [^{14}C]phosphonoformic acid binding, and Western blot analysis. Cisplatin inhibited phosphate uptake in a time- and dose-dependent manner, and also the Na^+-dependent uptake without altering Na^+-independent uptake. The cisplatin inhibition was not affected by the hydrogen peroxide scavenger catalase, but completely prevented by the hydroxyl radical scavenger dimethylthiourea. Antioxidants were ineffective in preventing the cisplatin-induced inhibition of phosphate uptake. Kinetic analysis indicated that cisplatin decreased Vmax of Na^+-dependent phosphate uptake without any change in the Km value. Na^+-dependent phosphonoformic acid binding was decreased by cisplatin treatment. Western blot analysis showed that cisplatin caused degradation of Na^+-dependent phosphate transporter protein. Taken together, these data suggest that cisplatin inhibits phosphate transport in renal proximal tubular cells through the reduction in the number of functional phosphate transport units. Such effects of cisplatin are mediated by production of hydroxyl radicals.

Key Words: Cisplatin, Na^+-Pi uptake, Kinetics, Phosphonoformic acid binding, NaP_i-4 protein expression, Opossum kidney cells

INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum II) is a very effective anticancer drug which is widely used in the treatment of the bladder, testis, ovary, and other solid tumors (Borch, 1987). The clinical usefulness is, however, limited by the development of nephrotoxicity, a side effect that may be produced in human and animals (Leonard et al, 1971; Madias & Harrington, 1978; Von Hoff et al, 1979; Garnick et al, 1988; Kim et al, 1995). Early clinical trials revealed that the incidence of nephrotoxicity ranges from 25~33% and 50~75% following single and multiple course therapy, respectively, with cisplatin (Lippman et al, 1973; Madias & Harrington, 1978). Our previous *in vivo* studies in rabbits have shown that cisplatin treatment causes an increase in inorganic phosphate excretion (Kim et al, 1995). However, the underlying mechanism has not been elucidated. Under normal conditions, 80~95% of the inorganic phosphate filtered through glomeruli are reabsorbed in the renal tubule, almost all of this occurring in the proximal tubule (Greger et al, 1977; Ullrich et al, 1977; Hammerman, 1986). In the present study, we investigated the effect of cisplatin on the inorganic phosphate uptake in renal proximal tubular epithelial cells using opossum kidney (OK) cells, an established proximal tubular cell line. OK cells possess many characteristics of proximal tubules and have been extensively used for studies on the regulation of inorganic phosphate transport in this segment (Caverzasio et al, 1986; Loghman-Adham & Motock, 1993; Mitchell, 1988).

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METHODS

Chemicals

[^{32}P]-phosphate and [^{14}C]phosphonoformic acid (PFA) were obtained from Amersham International (Amersham, UK). Deferoxamine (DFO), catalase, *N,N'*-diphenyl-*p*-phenylenediamine (DPPD), Tolox, dimethythiourea (DMTU), *t*-butylhydroperoxide (*t*BHP) and malondialdehyde tetraethylacetal were purchased from Sigma-Aldrich Chemical (St. Louis, MO). All other chemicals were of the highest

ABBREVIATIONS: DFO, deferoxamine; DMTU, dimethythiourea; DPPD, *N,N'*-diphenyl-*p*-phenylenediamine; Na^+-P_i, Na^+-dependent phosphate; NMG, *N*-methyl-D-glucamine; OK, opossum kidney; PFA, phosphonoformic acid; ROS, reactive oxygen species; *t*BHP, *t*-butylhydroperoxide.

commercial grade available.

Culture of OK cells

OK cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in 75-cm^2 culture flasks by serial passages (Costar, Cambridge, MA). The cells were grown in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12, Sigma Chemical Co.) containing 10% fetal bovine serum at 37°C in 95% air/5% CO_2 incubator. When the cultures reached confluence, subculture was prepared using a 0.02% EDTA-0.05% trypsin solution. All experiments started 3~4 days after plating when a confluent monolayer culture was achieved.

Uptake studies

The phosphate uptake was determined with cell monolayers grown on 24 well plates. After an exposure to cisplatin, the cells were washed twice with the uptake buffer containing the following (in mM): 137 NaCl, 5.4 KCl, 2.8 $CaCl_2$, 1.2 $MgSO_4$, and 10 Hepes (pH 7.4), and incubated for 30 min at 37°C in the uptake buffer containing 5 μM [^{32}P]-phosphate. For kinetic studies, the cells were incubated for 15 min at 37°C in the uptake buffer containing various concentrations (0.005~0.5 mM) of [^{32}P]-phosphate. For measurement of Na^+-independent phosphate uptake, NaCl was replaced by 137 mM *N*-methyl-D-glucamine (NMG). At the end of incubation period, the cells were washed three times with ice-cold uptake buffer and solubilized in 0.5 ml of 0.2% Triton X-100. Aliquots of each sample were transferred to scintillation vials and the radioactivity was counted in a liquid scintillation counter (TRI-CARB 2100TR, Packard, USA). Protein concentration was measured by the method of Bradford (Bradford, 1976).

[^{14}C]PFA binding studies

The binding of [^{14}C]PFA was measured in cell monolayers grown on 24 well plates. After an exposure to cisplatin, the cells were washed twice with the uptake buffer and incubated for 30 min at 37°C in the uptake buffer containing 1 mM [^{14}C]PFA. At the end of the incubation period, the cells were washed three times with ice-cold buffer and solubilized in 0.5 ml of 0.2% Triton X-100. Aliquots of each sample were transferred to scintillation vials and the radioactivity was counted in a liquid scintillation counter as described above.

Western blot

The intrinsic type II Na^+-dependent phosphate transporter protein (NaP_i-4) in OK cells was analyzed as described by Pfister et al. (Pfister et al, 1997). Cells were grown to confluence in 10-cm Petri dishes. After treatment with cisplatin, the cells were scraped off the dish and washed twice with TBS (0.9% NaCl and 10 mM Tris-HCl, pH 7.4). The scraped cells were homogenized in solution containing 10 mM NaCl, 1 mM EGTA, 1 mM EDTA, 20 mM Tris-HCl (pH 7.4), and 1% Triton X-100. The homogenate was centrifuged at 2,000 rpm for 10 min at 4°C. The supernatant was centrifuged again at 16,000 rpm for 40 min at 4°C. The pellet corresponding to a crude membrane preparation was resuspended in 100 μl of 50 mM mannitol and 10 mM Hepes-Tris (pH 7.2). Thirty μg of total protein were resolved by SDS-polyacrylamide gel electrophoresis (10%) and subsequently transferred to nitrocellulose membrane. Nonspecific binding was then blocked by incubating the membrane at room temperature for 1 hr in TBS containing 5% nonfat dry milk and 1% Triton X-100. The expression of the NaP_i-4 protein was estimated using an affinity pure polyclonal antibody (Alpha Diagnostic, Inc.) raised against C-terminal 12 amino acids of the published NaP_i-4 sequence (Sorribas et al, 1994) (antibody dilution, 1/100). Incubation with the primary antibody took place overnight at room temperature. The nitrocellulose membrane was washed four times with TBS containing 1% Triton X-100, and then incubated with a 1 : 10,000 dilution of an anti-rabbit IgG labeled with horseradish peroxidase (Amersham Life Science, Inc.) for 1 hr at room temperature. The membrane was washed four times with TBS containing 1% Triton X-100. Blots were developed by ECL kit.

Measurement of cell viability

The cell viability was estimated by a trypan blue exclusion assay. Cells were treated with cisplatin and harvested using 0.025% trypsin. Cells were then incubated with 4% trypan blue solution. Cells failing to exclude the dye were considered nonviable, and the data are expressed as percentage of nonviable cells.

Measurement of ATP content

ATP levels in OK cells were measured by a luciferin-luciferase assay method as described previously (Min et al, 2000).

Measurement of Na^+-K^+-ATPase activity

The Na^+-K^+-ATPase activity was assayed in the microsomal fraction prepared from OK cells. The ATPase activity was determined by measuring inorganic phosphate (Pi) released by ATP hydrolysis during the incubation of microsomes with an appropriate medium containing 3 mM ATP (Sigma) as a substrate. The total ATPase activity was determined in the presence of 100 mM Na^+, 20 mM K^+, 3 mM Mg^{2+}, 2 mM EDTA, and 40 mM imidazole (pH 7.4). The Mg^{2+}-ATPase activity was determined in the absence of K^+ and in the presence of 1 mM ouabain. The difference between the total and the Mg^{2+}-ATPase activities was taken as a measure of the Na^+-K^+-ATPase activity. At the end of a 10-min incubation, the reaction was terminated by the addition of 6% ice-cold perchloric acid. The mixture was then centrifuged at 3,500 g, and Pi in the supernatant was determined by the method of Fiske and SubbaRow (1925).

Statistical analysis

Data are expressed as mean±SEM. Comparisons between two groups were made using the unpaired *t* test. Multiple group comparisons were done using one-way analysis of variance followed by the Tukey *post hoc* test. $p<0.05$ was considered statistically significant.

RESULTS

To examine the effect of cisplatin on Na^+-dependent

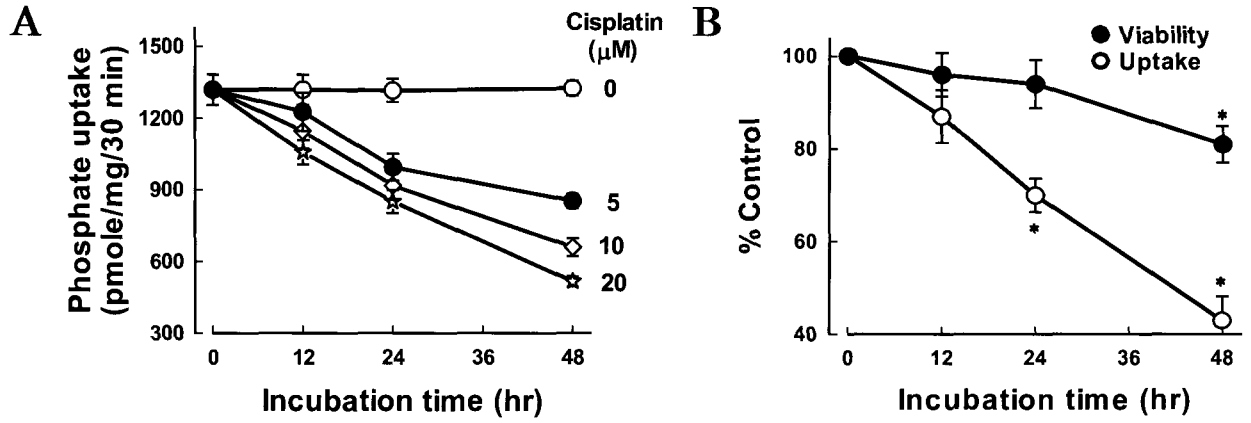

Fig. 1. (A) Effect of cisplatin on phosphate uptake in OK cells. Cells were exposed to various concentrations of cisplatin for 12~48 hr, and the uptake was measured for 30 min. Data are mean±SE of four experiments. (B) Effect of cisplatin on phosphate uptake and cell viability. Cells were exposed to 10 μM cisplatin for 12~48 hr. The uptake was measured for 30 min and cell viability was measured by trypan blue exclusion. Data are mean±SE of four experiments. *$p<0.05$ compared with control without cisplatin.

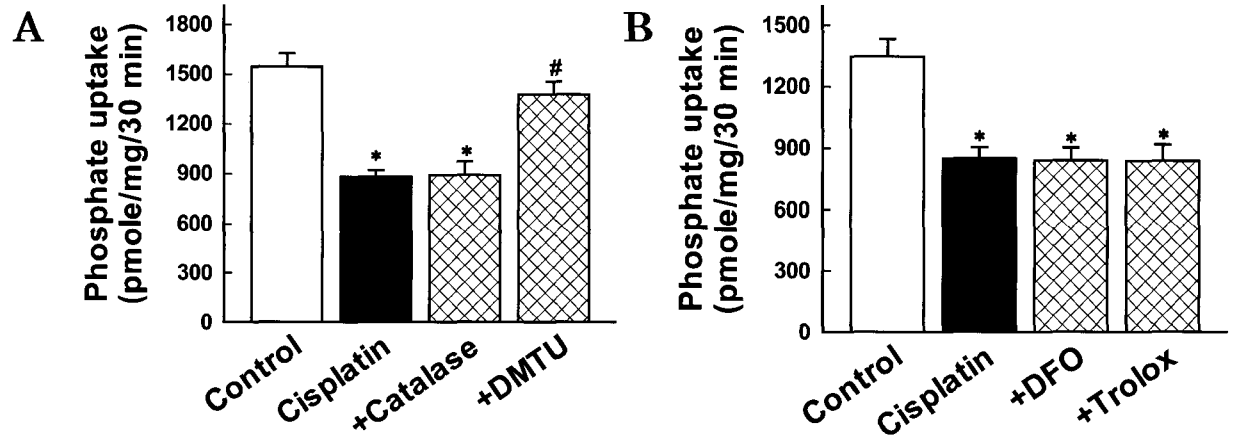

Fig. 2. (A) Effects of radical scavengers on cisplatin-induced inhibition of phosphate uptake in OK cells. Cells were exposed to 10 μM cisplatin for 48 hr in the presence of 500 units/ml catalase and 30 mM dimethythiourea (DMTU). The uptake was measured for 30 min. Data are mean±SE of five experiments. *$p<0.05$ compared with control; #$p<0.05$ compared with cisplatin alone. (B) Effects of antioxidants on cisplatin-induced inhibition of phosphate uptake in OK cells. Cells were exposed to 10 μM cisplatin for 48 hr in the presence of 0.2 mM deferoxamine (DFO) and 1 mM Trolox. The uptake was measured for 30 min. Data are mean±SE of five experiments. *$p<0.05$ compared with control.

phosphate uptake in OK cells, cells were exposed to 5, 10, and 20 μM cisplatin for 12, 14, and 48 hr. After the exposure, the uptake was determined for 30 min in the normal medium without cisplatin. As shown in Fig. 1A, cisplatin reduced the phosphate uptake in a time- and dose-dependent manner. When cells were exposed to each concentration of cisplatin for 48 hr, the uptake was reduced to 64, 50, and 42% of control, respectively. In subsequent experiments, therefore, OK cells were treated with 10 μM cisplatin for 48 hr.

To determine whether the inhibition of Na^+-dependent phosphate uptake was attributed to irreversible cell injury, we investigated the effect of cisplatin on cell death by measuring trypan blue exclusion. Cisplatin caused cell death in a dose-dependent manner. However, the extent of cell death was much lower than that of the uptake. Thus, when cells were exposed to 10 μM cisplatin for 48 hr, the uptake was inhibited to approximately 40% of the control, whereas approximately 19% of cells lost the viability (Fig. 1B).

Cisplatin inhibited the phosphate uptake in the presence of Na^+, whereas it did not affect the uptake in the absence of Na^+ (1,121.13±21.87 vs. 653.63±62.72 pmole/mg/30 min in the presence of Na^+; 182.02±33.54 vs. 204.38±20.42 pmole/mg/30 min in the absence of Na^+), indicating that cisplatin inhibits the active phosphate uptake driven by the gradient of Na^+ without any effect on Na^+-independent passive uptake. In the control cells, the uptake in the absence of Na^+ was approximately 16% of the total uptake. Therefore, the Na^+-independent uptake was not routinely measured in the subsequent experiments.

To investigate the role of reactive oxygen species (ROS) in the cisplatin-induced inhibition of Na^+-dependent phosphate uptake, the effects of radical scavengers were

examined. Catalase, a hydrogen peroxide scavenger, did not affect the inhibition of Na^+-dependent phosphate uptake induced by cisplatin, whereas DMTU, a hydroxyl radical scavenger, exerted a significant protective effect (Fig. 2A). These results suggest that hydroxyl radicals but not H_2O_2 are responsible for the Na^+-dependent uptake inhibition.

Since hydroxyl radicals are a potent initiator of lipid peroxidation (Koppenol, 1993), the cisplatin-induced inhibition of Na^+-dependent phosphate uptake could result from lipid peroxidation. To test this possibility, the effects of antioxidants were examined. DFO, an iron chelator, and Trolox, a water-soluble vitamin E compound, did not prevent the cisplatin-induced inhibition of Na^+-dependent phosphate uptake (Fig. 2B). A lipid-soluble antioxidant, DPPD, was also ineffective in the 5~100 μM concentration range. However, the inhibition of the uptake by inorganic hydroperoxide *t*BHP, which was employed as a positive control, was significantly prevented by 10 μM DPPD (Table 1). These data suggest that the effect of cisplatin on phosphate uptake is not mediated by lipid peroxidation.

In an attempt to gain insight into the mechanism by which cisplatin modulates phosphate uptake, the kinetic analysis of phosphate uptake was performed in cells treated with cisplatin. Since phosphate uptake was linear up to 20-min incubation in both control and cisplatin-treated cells (Fig. 3A), the kinetics of phosphate uptake was analyzed by measuring the initial velocity (15 min) of phosphate uptake as a function of phosphate concentration in the presence or absence of external Na^+. The Na^+-dependent uptake, computed by subtracting the uptake in the absence of Na^+ from the total uptake in each group, was kinetically analyzed. An Eadie-Hofstee transformation of the Na^+-dependent uptake showed that the relationship between the initial rate of phosphate uptake (V) and V/[phosphate] was linear in both control and cisplatin-treated cells (Fig. 3B). This indicates that in both cases the Na^+-dependent phosphate uptake follows a simple Michaelis-Menten kinetics, i.e., $V=Vmax\times(S)/[Km+(S)]$, where Vmax is the maximal uptake, (S) is the substrate concentration, and Km is the apparent Michaelis constant indicating the concentration of phosphate for 1/2Vmax. Cisplatin caused a significant reduction in the Vmax for Na^+-dependent phosphate uptake (5.48±0.10 vs. 12.05±0.03 pmole/mg/15 min in control cells), with no significant change in the apparent Km (0.054±0.009 vs. 0.042±0.004 mM in control cells).

PFA acts as a specific and competitive inhibitor of Na^+-dependent phosphate transport across the renal brush-border membrane and has been employed as a probe in studies of this transport system (Szczepanska et al, 1986; Szczepanska et al, 1989; Hoppe et al, 1991), including detection of changes in the number of Na^+-dependent phosphate transporters in membrane occurring in response to various stimuli (Yusufi et al, 1989; Hoppe et al, 1991). Therefore, we examined the effect of cisplatin on PFA binding in OK cells, and the results are depicted in Fig. 4. Total PFA binding measured in the presence of external Na^+ was significantly reduced by cisplatin, whereas the binding in the absence of external Na^+ was not different between the control and cisplatin-treated cells (Fig. 4A).

Table 1. Effect of *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) on reduced phosphate uptake induced by cisplatin and *t*-butyl-hydroperoxide (*t*BHP) in OK cells

Conditions	Uptake (pmole/mg/30 min)
Control	1,544.67±82.86
Cisplatin	880.67±40.81*
+DPPD (5 μM)	894.38±166.51*
+DPPD (10 μM)	851.13±135.28*
+DPPD (50 μM)	850.63±106.72*
+DPPD (100 μM)	866.00±106.11*
*t*BHP	722.48±73.28*
+DPPD (10 μM)	1,317.65±86.24#

Cells were exposed to 10 μM cisplatin for 48 hr or 0.3 mM *t*BHP for 2 hr in the presence or absence of DPPD. The uptake was measured for 30 min in the normal medium without any agents. Data are mean±SE of four determinations.

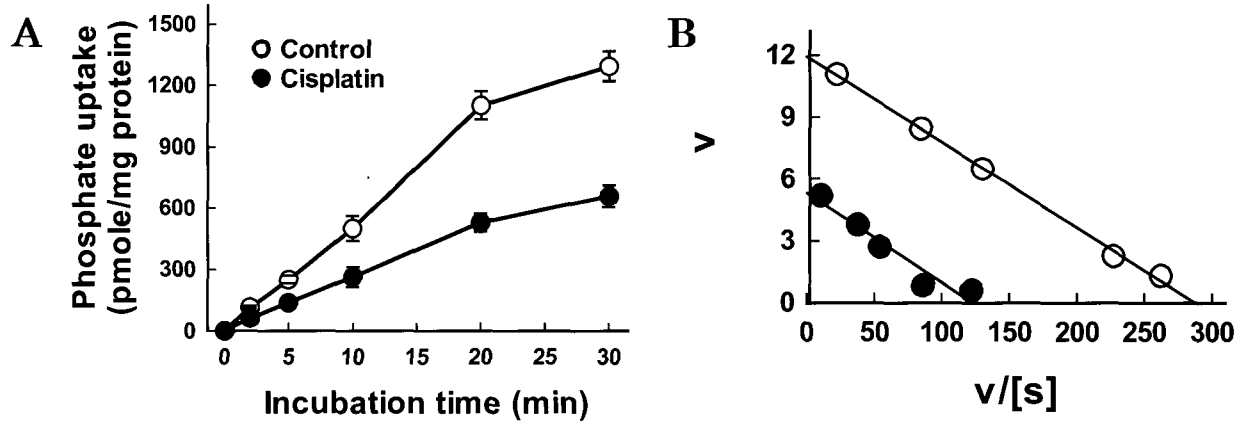

Fig. 3. (A) Initial rate of phosphate uptake in control and cisplatin-treated cells as a function of phosphate concentrations. Cells were exposed to 10 μM cisplatin for 48 hr and the uptake was measured for various time points (2~30 min). (B) Kinetic analysis of Na^+-dependent phosphate uptake. Cells were exposed to 10 μM cisplatin for 48 hr and the uptake was measured for 15 min in a buffer with or without Na^+ (replaced by NMG). Na^+-dependent uptake was calculated by subtracting the uptake in the absence of Na^+ from the total uptake in the presence of Na^+. The results were plotted according to Eadie-Hofstee transformation of Michaelis-Menten equation. In this plot, the intercept of the line with Y-axis represents Vmax and the slope indicates the Km for phosphate.

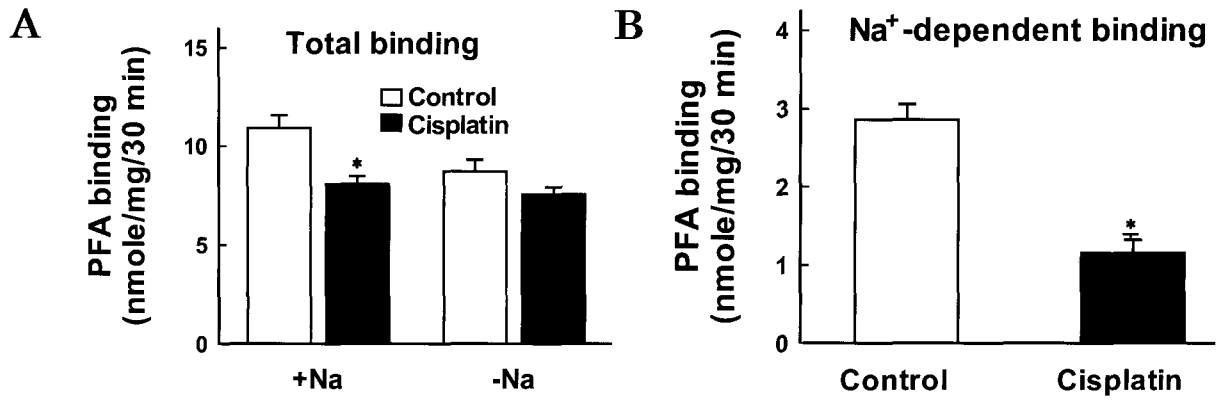

Fig. 4. Effect of cisplatin on phosphonoformic acid (PFA) binding in OK cells. Cells were exposed to 10 μM cisplatin for 48 hr. Total PFA binding (A) was measured for 30 min in a buffer with or without Na^+ (replaced by NMG). Na^+-dependent binding (B) was calculated by subtracting the binding in the absence of Na^+ from the total binding in the presence of Na^+. Data are mean±SE of five experiments. *$p<0.05$ compared with control.

Therefore, cisplatin induced a significant reduction in the Na^+-dependent PFA binding (Fig. 4B).

Expression of NaP_i-4 protein in OK cells was also analyzed by Western blot. Fig. 5 shows that treatment with cisplatin for 24 and 48 h caused a time-dependent decrease of NaP_i-4 protein. Such changes were prevented by DMTU, but not by catalase and Trolox. Taken together with data from kinetic analysis and PFA binding assay, these results suggest that the cisplatin-induced inhibition of Na^+-dependent phosphate uptake is due to a decrease in the number of phosphate transporters.

In the last series of experiments, we determined whether cisplatin inhibited cellular ATP content and Na^+-K^+-ATPase activity at concentrations which caused a significant reduction in phosphate uptake. As shown in Table 2, cisplatin did not alter the cellular ATP content and Na^+-K^+-ATPase activity in microsomal fraction prepared from OK cells.

Table 2. Effect of cisplatin on cellular ATP content and Na^+-K^+-ATPase activity in OK cells

Conditions	ATP content (nmole/mg protein)	Na^+-K^+-ATPase activity (μmole Pi/mg protein/hr)
Control	11.67±2.86	1.67±0.46
Cisplatin	10.85±3.23	1.54±0.23

Cells were exposed to 10 (M cisplatin for 48 hr. The cellular ATP content and Na^+-K^+-ATPase activity were measured as described in "METHODS". Data are mean±SE of four determinations.

DISCUSSION

In the kidney, reabsorption of filtered inorganic phosphate takes places along the proximal tubules and is controlled by a variety of hormones and other factors. Three structurally unrelated Na^+-dependent phosphate transporter families (Type I, Type II, and Type III) have been identified (Murer et al, 2000). The type II family can further be subdivided into two groups, type IIa and type IIb, with an overall homology of ~60% and the major differences at the COOH terminus between both groups (Hilfiker et al, 1998). Immunohistochemistry reveals that the type IIa is located in the apical membrane of proximal tubular cells and is largely responsible for renal Na^+-dependent inorganic phosphate reabsorption (Murer et al, 2000). Most of the proximal tubular characteristics involved in inorganic phosphate handling are retained in OK cells. These cells contain an endogenous Na^+-dependent inorganic phosphate transporter apically located and regulated by the same hormones and factors as type IIa transporters in proximal tubules (Pfister et al, 1997; Pfister et al, 1998).

Most of the studies on cisplatin nephrotoxicity have been focused on cell death, however, relatively little information is available regarding the effect of cisplatin on membrane transport function with special reference to inorganic phosphate transport. Cisplatin can cause either necrosis or apoptosis of renal epithelial cells. The form of cell death has been demonstrated to depend on the concentration of cisplatin: high concentrations (>200 μM) lead to necrosis and lower concentrations (<100 μM) induce exclusively apoptosis (Lieberthal et al, 1996; Cummings & Schnellmann, 2002; Kim et al, 2005). At a therapeutic dose (80 mg/m^2), plasma platinum levels ranged from 10 to 23 μM 24 h after a singe cisplatin infusion (Campbell et al, 1983; Dumas et al, 1985). Based on these data, therefore, we exposed the cells to 10 μM cisplatin for 48 hr.

In the present study, we demonstrated that Na^+-dependent inorganic phosphate transport was inhibited by cisplatin treatment in a concentration- and time-dependent manner (Fig. 1A). Although cisplatin also reduced cell viability, the extent of the uptake inhibition was much greater than the loss of cell viability (Fig. 1B), suggesting that most of the uptake inhibition are not a result of irreversible cell death.

Although cisplatin-induced apoptosis in various cell types has been reported to be mediated by ROS generation (Lieberthal et al, 1996; Ikeda et al, 1999; Wang et al, 2000), the chemical nature of ROS remains to be clarified. Also, the role of ROS in cisplatin-induced inhibition of membrane transport function is not clear. In the present study, the

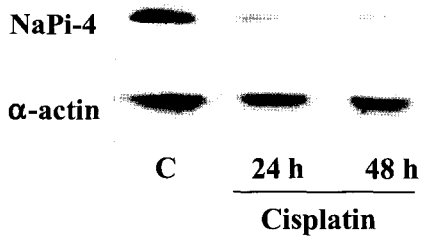

Fig. 5. Effect of cisplatin on expression of the Na^+-dependent phosphate transporter (NaP_i-4) protein. Cells were exposed to 10 μM cisplatin for 24 or 48 h, and the expression of NaP_i-4 protein was analyzed by Western blot.

cisplatin-induced inhibition of Na^+-dependent phosphate uptake was completely prevented by the hydroxyl radical scavenger DMTU, but not by the H_2O_2 scavenger catalase (Fig. 2A). These data are consistent with previous studies that cisplatin directly increases hydroxyl radical formation in renal epithelial cells (Baliga et al, 1998), and that cisplatin-induced apoptosis in LLC-PK_1 cells is prevented by the hydroxyl radical scavengers (Lieberthal et al, 1996). Because hydroxyl radicals are a potent initiator of lipid peroxidation (Koppenol, 1993), the inhibition of Na^+-dependent phosphate uptake induced by cisplatin could be resulted from lipid peroxidation. However, the cisplatin inhibition was not altered by antioxidants (Fig. 2B, Table 1). These findings suggest that cisplatin inhibits Na^+-dependent phosphate uptake through a lipid peroxidation-independent mechanism.

In the present study, the Vmax of Na^+-dependent phosphate uptake was significantly reduced in cells treated with cisplatin, whereas the Km value remained unchanged (Fig. 3). In the kinetic analysis of carrier-mediated transport, the Vmax is determined by two factors: firstly, the capacity of the carrier system and, secondly, the proportion of adsorbed molecules which dissociate in a forward direction in a unit time. The former depends mainly on the number of carrier sites per unit area of membrane, and the latter depends on (1) the probability of a substrate molecule to dissociate from a carrier site in a given time, and (2) the rate of turnover of carrier across the membrane (Neame & Richards, 1972). Since the Km for phosphate in the present study was not changed, it is unlikely to have altered carrier-substrate dissociation. Therefore, the decrease in Vmax could be attributed to reduction in the number of functional carrier units or the rate of turnover. Assuming that PFA binding provides an accurate estimate of the number of Na^+-Pi transport (Szczepanska et al, 1987; Yusufi et al, 1989), the results of PFA binding studies (Fig. 3) indicate that the decrease in Vmax may be mediated by a decrease in the number of functional carrier units. These results were further supported by Western blot analysis (Fig. 5). The effects of cisplatin on kinetic parameters are consistent with actions of other toxic compounds such as cadmium (Park et al, 1997), $HgCl_2$ (Loghman-Adham, 1992), and gentamicin (Levi & Cronin, 1990) in renal brush-border membrane vesicles. By contrast, Courjault-Gautier et al. (1994) reported in primary cultures of rabbit proximal tubular cells that treatment of 50 μM cisplatin for 24 h causes a decrease in Km value without altering Vmax of Na^+-dependent phosphate uptake. This discrepancy may be attributed to differences in species and cisplatin concentration used.

Since phosphate is taken up by Na^+-phosphate cotransport system in the renal proximal tubular cells, maintenance of intracellular Na^+ gradient needs to drive phosphate transport. The Na^+ gradient across plasma membrane is generated by the active Na^+ pump in the basolateral membrane. Thus, the cisplatin-induced inhibition of phosphate uptake in the present study could be a consequence of a decrease in the cellular ATP content and the Na^+-K^+-ATPase activity in the basolateral membrane. However, the present study showed that the cisplatin-induced reduction of phosphate uptake did not result from reduced ATP content and the inhibition of Na^+-K^+-ATPase activity.

In summary, cisplatin inhibited Na^+-dependent phosphate uptake in OK cells through a reduction in the number of transporter unit. Generation of hydroxyl radicals might be involved in the cisplatin-induced inhibition through a lipid peroxidation-independent mechanism.

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