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Effect of Cisplatin on Na^+/H^+ Antiport in the OK Renal Epithelial Cell Line

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Cis-diamminedichloroplatinum II (cisplatin), an effective antitumor agent, induces acute renal failure by unknown mechanisms. To investigate direct toxic effects of cisplatin in the renal proximal tubular transport system, OK cell line was selected as a cell model and Na^+/H^+ antiport activity was evaluated during a course of cisplatin treatment. The cells grown to confluence were treated with cisplatin for 1 hour, washed, and incubated for up to 48 hours. At appropriate intervals, cells were examined for Na^+/H^+ antiport activity by measuring the recovery of intracellular pH (pHi) after acid loading. Cisplatin of less than 50 μM induced no significant changes in cell viability in 24 hours, but it decreased the viability markedly after 48 hours. In cells exposed to 50 μM cisplatin for 24 hours, the Na^+-dependent pHi recovery (i.e., Na^+/H^+ antiport) was drastically inhibited with no changes in the Na^+-independent recovery. Kinetic analysis of the Na^+-dependent pHi recovery indicated that the Vmax was reduced, but the apparent Km was not altered. The cellular Na^+ and K^+ contents determined immediately before the transport measurement appeared to be similar in the control and cisplatin group, thus, the driving force for Na^+-coupled transport was not different. These results indicate that cisplatin exposure impairs the Na^+/H^+ antiport capacity in OK cells. It is, therefore, possible that in patients treated with a high dose of cisplatin, proximal tubular mechanism for proton secretion (hence HCO_3^- reabsorption) could be attenuated, leading to a metabolic acidosis (proximal renal tubular acidosis).

Key Words: Cisplatin, OK cell, Na^+/H^+ antiport

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) is highly effective antitumor agent (Roseneweig et al, 1977), but its therapeutic usefulness is limited by its pronounced nephrotoxicity (Safirstein et al, 1986). Several authors have described the effects of this drug on the renal structure and functions (Dentino et al, 1978; Dobyan et al, 1980; Daugaard et al, 1987). Reduction in glomerular filtration rate (Winston & Safirstein, 1985; Daugaard et al, 1988), impaired urine concentrating ability (Safirstein et al, 1982; 1986), renal potassium and magnesium wasting (Schilsky & Anderson, 1979; Blachley & Hill, 1981; Mavichak et al, 1985), diminished proximal tubular sodium and fluid reabsorption (Daugaard et al, 1986), and defect in proximal tubular acidification (Lacchini et al, 1992) are among the important findings.

The mechanisms by which cisplatin induces these lesions are not completely understood. With respect to the cisplatin effect on urine acidification, renal tubular stopped-flow, microperfusion experiments in rats by Lacchini et al (1992) have indicated that cisplatin treatment inhibits urine acidification at the proximal tubule without increasing H^+ back leakage across the tubule. Such results may suggest that the Na^+/H^+ antiporter activity is impaired by cisplatin, as the proximal tubular acidification is primarily determined by secretion of H^+ via the Na^+/H^+ antiporter (Malnic, 1987; Ross, 1989). The present study

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was, therefore, undertaken to examine this possibility. OK cell line was selected as a model system to evaluate direct cellular effects of cisplatin on the Na^+/H^+ antiporter. The OK cell is a continuous cell line derived from American opossum kidney (Koyama et al, 1978). When grown to confluence, these cells express many characteristics of proximal tubular epithelia, including various sodium-dependent secondary active transports (Malmstrom & Murer, 1986; Malmstrom et al, 1987; Quamme et al, 1989; Van den Bosch et al, 1989; Montrose & Murer, 1990a).

METHODS

Cell culture

OK cell line was obtained from the American Type Culture Collection (ATCC) and maintained by serial passages in 75 cm^2 plastic culture flasks (Corning, New York). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum and 1% antibiotic/antimycotic solution (Gibco) in an atmosphere of 5% CO_2-95% air at 37°C (Montrose & Murer, 1990a). The culture was fed with fresh medium every 3 days. When cell growth reached saturation density, subcultures were prepared by treatment with 0.5% trypsin-0.2% ethylenediaminetetraacetic acid (EDTA) in Ca^{2+} and Mg^{2+}-free Hank's balanced salt solution (HBSS, Gibco) for 5~10 min at 37°C. The cells were suspended by gentle shaking of the culture flasks and then plated at 1:6 dilution with culture medium in 75 cm^2 plastic culture flasks. Confluence occurred after 3~4 days in culture. The cell monolayers were maintained in serum-free medium for 24 hours before drug treatment. All experiments were performed on passages 80~95.

Cisplatin treatment

Cisplatin was dissolved in a sterile salt medium containing (in mM) 114 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.2 $CaCl_2$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 16 $NaHCO_3$, and 5.5 glucose, saturated with 5% CO_2-95% air (pH 7.4) at 37°C. Cell monolayers were washed twice with HBSS and then exposed to cisplatin-containing medium for 60 min. Upon completion of exposure the monolayers were washed three times with HBSS to remove the residual drugs and were incubated in DMEM containing 5% fetal bovine serum (Baldew et al, 1992). In control experiments, monolayers were exposed to the vehicle (plain salt medium).

Determination of cell viability

Monolayers of OK cells were detached by incubating them in Ca^{2+} and Mg^{2+}-free HBSS containing 0.5% trypsin and 0.2% EDTA for 5~10 min at 37°C. The viability was assessed by counting viable cells in the presence of trypan blue using hemocytometer and quantified by measuring the amount of protein in the viable cells (Baldew et al, 1992). The protein concentration was determined by the method of Bradford (1976) using the Bio-Rad Protein Assay Kit with bovine γ-globulin as a standard.

Measurement of intracellular pH (pHi) recovery from an acid load

As a measure of Na^+/H^+ antiport activity the Na^+-dependent recovery of intracellular pH (pHi) was determined in acid loaded OK cells. The pHi recovery was determined both in the presence and absence of Na^+ in the extracellular medium, and the difference was taken as the Na^+-dependent component. The Na^+-containing medium was Hank's salt solution (HSS) consisting of (in mM) 140 NaCl, 4.2 $NaHCO_3$, 0.36 Na_2HPO_4, 0.5 $MgCl_2$, 1.3 $CaCl_2$, 5.4 KCl, 0.44 KH_2PO_4, and 5.5 glucose and 10 Hepes, pH 7.2. In Na^+-free HSS NaCl was replaced by equimolar concentration of TMA-chloride, and $NaHCO_3$ and Na_2HPO_4 were replaced by respective K^+-salts. OK cell monolayers were detached with trypsin as described above and the cell suspension was centrifuged at 1,100 rpm for 5 min. The cells were then washed twice with Na^+-free HSS by centrifuging at 1,100 rpm for 5 min. The washed cells were resuspended in Na^+-free HSS and equilibrated for 15 min at 37°C.

Changes in pHi was monitored using a pH-sensitive fluorescent dye 2',7'-bis-(2-carboxyethyl)-5,6-carboxyfluorescein (BCECF). Cells were first loaded with the dye by incubating them in Na^+-free HSS containing 3 μM triacetoxymethyl ester of BCECF (BCECF-AM) for 30 min at 37°C. BCECF-AM was added from a 1 mM stock solution in dimethylsulfoxide. After entering the cell BCECF-AM is hydrolyzed by esterase, leaving free BCECF in the cell. The BCECF-loaded cells were washed twice with

dye-free Na^+-free HSS by centrifuging at 1,100 rpm for 5 min and resuspended in Na^+-free HSS. The pHi of the cell containing BCECF was determined by a spectrofluorometer as described by Montrose et al, (1987) and Montrose and Murer (1990b). A preliminary experiment was carried out to obtain appropriate dual excitation wavelengths. Samples were excited at various wavelengths between 400~550 nm and the emission was recorded at 530 nm. The value of emission was then plotted against the excitation wavelength and found 450 nm as the excitation wavelength for cellular autofluorescence and 505 nm as that for maximal BCECF fluorescence emission (Fig. 1A). Thus, the ratio of emissions at excitation wavelengths 505 and 450 nm was recorded as the BCECF fluorescence corrected for cellular autofluorescence. The ratio was then converted to pHi value using a calibration curve. The calibration curve for OK cells (Fig. 1B) was constructed by determining BCECF fluorescence ratios in solutions (110 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 30 mM choline chloride, and 20 mM Hepes) of varying pH (6.0~8.0) containing nigericin (10 μM), a protonophore (Dagher & Sauterey, 1987).

Acid loading to the cell was achieved by ammonium chloride prepulse technique (Guggino et al, 1983). In this technique, cells are exposed to Na^+-free HSS containing 25 mM NH_4Cl at 37°C for 10 min, during which the NH_3 moved into the cell is converted to NH_4^+, increasing the pHi. When these cells are exposed to NH_4Cl-free solution, the NH_3 dissociated from NH_4 quickly moves out of the cell by non-ionic diffusion, leaving H^+ in the cell. In practice, at the end of incubation with NH_4Cl, an aliquot (~20 μl) of cell suspension is diluted with a large volume (2 ml) of NH_4Cl-free solution (either HSS or Na^+-free HSS) in a cuvette.

Measurement of cellular electrolytes

Cells were suspended in Na^+-free HSS containing ^{3}H-inulin (10 μCi/ml). The cell suspensions were centrifuged at 5,000 rpm for 10 min. The supernatants were removed and the packed cells were extracted for 5 hours in an equal volume of concentrated nitric acid (13.6 M). Na^+ and K^+ in the extracts were measured with a flame photometer (Radiometer, model FLM3, Copenhagen, Denmark) and ^{3}H activities with a liquid scintillation counter (Packard, Tricarb 4530). The Na^+ and K^+ contents of packed cells were corrected for the contamination by extracellular fluid (^{3}H-inulin space) and expressed as mmole/*l* cell.

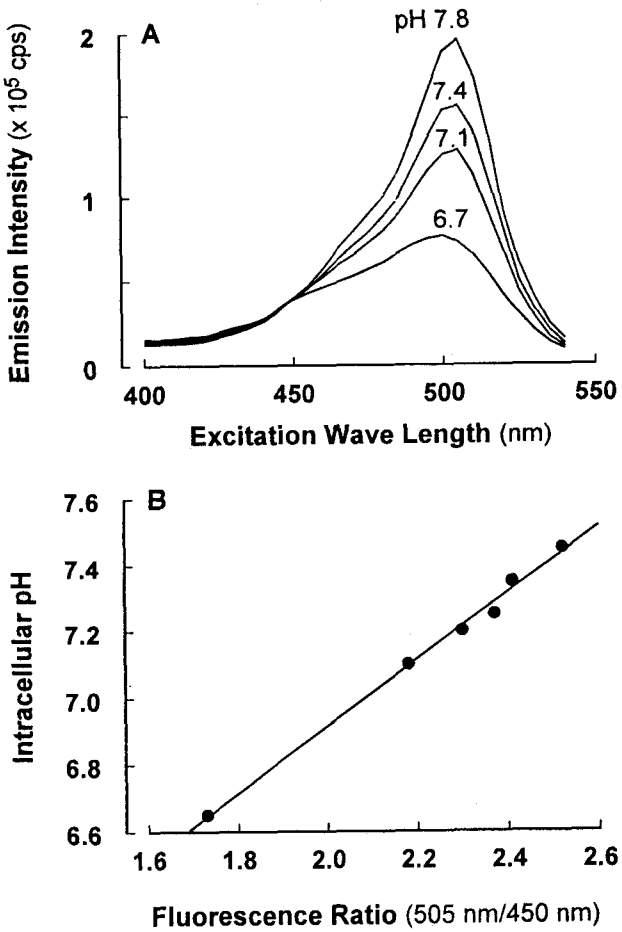

Fig. 1. A: Changes in emission intensity according to the excitation wave length in spectrophotometry of BCECF-loaded OK cells suspended in media of various pH. B: The calibration curve for intracellular pH in spectrophotometry of BCECF-loaded OK cells. The ratio of emission intensities (Fluorescence ratio) determined at the excitation wave lengths of 505 and 450 nm was plotted against the pH of suspending medium containing 10 μM nigericin, a protonophore.

Chemicals and materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, trypsin, and antibacterial/antimycotic solution were purchased from Gibco (Grand Island, NY, USA). We obtained 2',7'-bis-(2-carboxyethyl)-5,6-carboxyfluorescein (BCECF)-triacetoxymethyl ester and ethylisopropyl amiloride (EIPA) from Molecular Probes (Eugene, ORS), ^{3}H-inulin from New England Nuclear (Boston, MA, USA), and

cisplatin from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were of analytical grade.

Statistical analysis

Statistical evaluation of data was done using Student's t-test and covariance analysis. Differences with $p<0.05$ were considered statistically significant.

RESULTS

Effect of cisplatin on OK cell viability

The first series of experiments was on the time and concentration dependence of OK cell viability on cisplatin exposure. The cell monolayers were exposed to 10, 50, or 100 μM cisplatin for 1 h, washed and incubated with fresh medium for 24 or 48 h before the test. In cells exposed to 10 μM cisplatin, there was no substantial change in viability during 48 h. In cells exposed to 50 μM cisplatin, viability decreased slightly (~4%), but not significantly, during the first 24 h, but it fell significantly (~32%) after 48 h. In cells exposed to 100 μM cisplatin, viability decreased significantly both at 24 h (~22 %) and 48 h (~57%) after the drug exposure.

Effect of cisplatin on Na^+/H^+ exchange in OK cell

Fig. 2A shows a typical tracing of pHi changes determined by BCECF fluorescence technique in acid-loaded OK cells. Upon intracellular acid loading the pHi dropped to about 6.8 and then recovered slowly as H^+ ions moved out. The heavy line in the center of the fluorescence tracing is the regression line generated by computer fitting of data using the following equation:

$$f(t)=a+b\cdot e^{-ct}$$

where, a, b and c are constant, t is time, and f(t) is fluorescence at time t. The initial velocity of pHi change is obtained from the derivative of the function f(t) at t=0.

Fig. 2B depicts the time courses of pHi recovery in the presence and absence of sodium in the extracellular medium. For simplicity, only the regression lines are illustrated. The upper most line represents the data for sodium (140 mM)-containing medium.

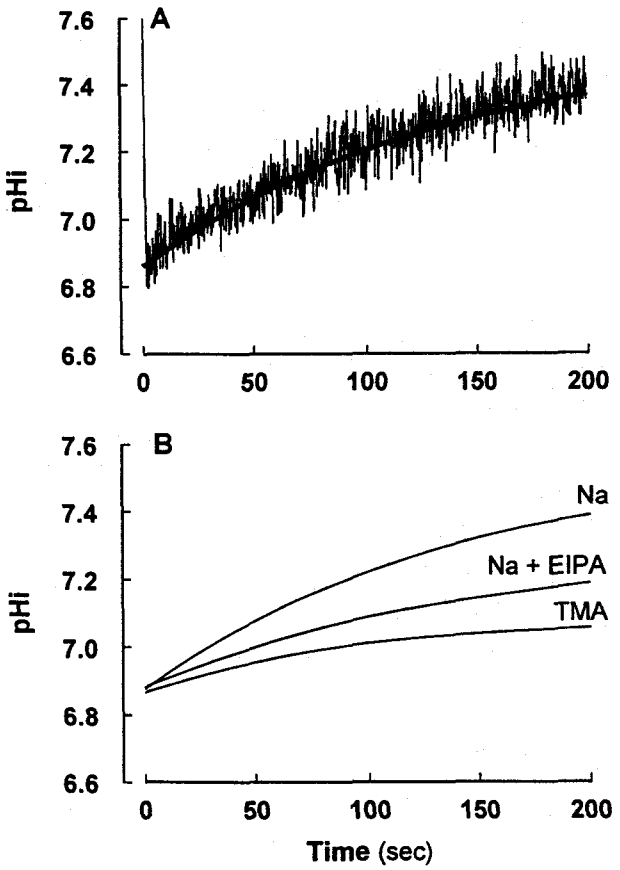

Fig. 2. A: A typical tracing of pHi response to an intracellular acid load in the OK cell. Acid loading by NH_4Cl prepulse technique causes a quick drop of pHi to about 6.8. The pHi is then slowly reverted as H^+ ions move out of the cell. The line in the center of the flores-cence tracing is the regression line generated by computer fitting of the data using the equation $f(t)=a+be^{-ct}$. B: Time courses of pHi recovery of acid-loaded OK cells in Na^+-, TMA-, or Na^+ and EIPA-containing media. For simplicity, only the regression lines of fluorescence tracings are depicted. The concentration of Na^+ and TMA (tetramethylammonium) was 140 mM and that of EIPA (ethylisopropyl amiloride) was 100 μM. Data repre-ent a typical experiment.

When sodium was replaced by tetramethylammonium (TMA), the rate of pHi recovery was markedly reduced. The difference between these two lines represents the sodium-dependent pHi recovery, which may be mediated by Na^+/H^+ antiporter, as it was inhibitable by 100 μM ethylisopropyl amiloride (EIPA), a specific inhibitor of renal Na^+/H^+ anti-porter (Helmle-Kolb et al, 1990). These results confirm the existence of Na^+/H^+ antiport system in

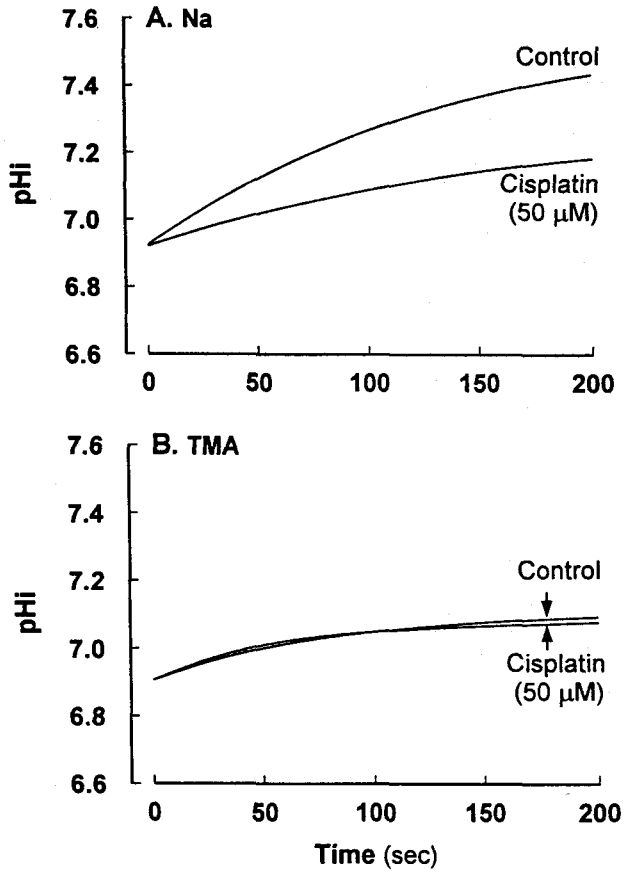

Fig. 3. Time courses of pHi recovery of acid-loaded control and cisplatin-treated OK cells in Na^+-containing (Panel A) and Na^+-free (Panel B) media. For simplicity, only the regression lines of fluorescence tracings are depicted. In cisplatin group, the cell monolayers were treated with 50 μM cisplatin for 1 h, washed, and incubated for 24 h before they were tested for pHi recovery. Data represent a typical experiment.

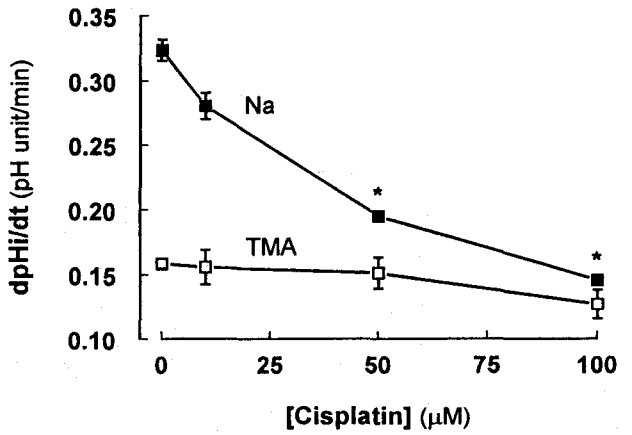

Fig. 4. Effect of cisplatin treatment on the initial rate of pHi recovery (dpHi/dt) in Na^+-containing and Na^+-free (TMA) media as a function of cisplatin concentration. The value of dpHi/dt was obtained by taking the derivative of the function $f(t)=a+be^{-ct}$ at $t=0$. In cisplatin groups, cell monolayers were exposed to cisplatin of indicated concentrations, washed, and incubated for 24 h before the test of pHi recovery. Data represent mean ± SE of 3 determinations. *Significantly different from the control (0 μM cisplatin) value.

Table 1. Sodium and potassium contents of the control and cisplatin-treated OK cells

	$[Na^+]$ (mEq/*l* cell)	$[K^+]$
Control	14.59 ± 1.11	10.44 ± 0.71
Cisplatin	14.35 ± 1.05	9.41 ± 0.25

Cells were prepared in a way similar (except BCECF loading) to that for the pHi recovery study. Cells were preincubated for 30 min in Na^+-free (TMA)-medium, then suspended in TMA-medium containing NH_4Cl (25 mM) and ^3H-inulin, and analyzed for Na^+, K^+ and inulin. The Na^+ and K^+ contents of the packed cells were corrected for the contamination by extracellular fluid using inulin space.

OK cells, as reported by others (Gennari et al, 1992; Murer et al, 1994).

Fig. 3 compares time courses of pHi recovery between the control and cisplatin (50 μM)-treated cells. In cisplatin-treated cells, the pHi recovery in sodium-containing medium was significantly retarded (Fig. 3A), but that in sodium-free medium was not different from the control (Fig. 3B), indicating that the Na^+/H^+ antiport activity was suppressed.

The Na^+ content in the cell immediately before the pHi measurement was comparable in the control and cisplatin group (Table 1), indicating that the imposed Na^+ gradient (i.e., the initial driving force for Na^+/H^+ antiport) was identical.

Fig. 4 shows changes in the initial rate of pHi recovery (dpHi/dt) in cisplatin-treated cells as a function of cisplatin concentration. The dpHi/dt in sodium-containing medium decreased progressively as the cisplatin concentration increased, whereas that in the sodium-free (TMA) medium remained unchanged at all cisplatin concentrations. Consequently, the sodium-dependent dpHi/dt appeared to be progressively reduced as the cisplatin concentration increased. The average reduction was 25% with 10

μM cisplatin, 73% with 50 μM cisplatin, and 90% with 100 μM cisplatin.

Fig. 5 shows the effect of cisplatin treatment on Na^+/H^+ antiport kinetics. In the upper panel sodium-dependent dpHi/dt for the control and cisplatin (50 μM)-treated cells are plotted against the sodium concentration in the medium, and in the lower panel the same data are depicted as Hofstee plots. It is evident that in cisplatin-treated cells the Vmax was markedly reduced (0.225 in control vs. 0.081 in cisplatin group), but the Km was not significantly changed (31.0 mM in control vs. 29.6 in cisplatin group) as compared with the control.

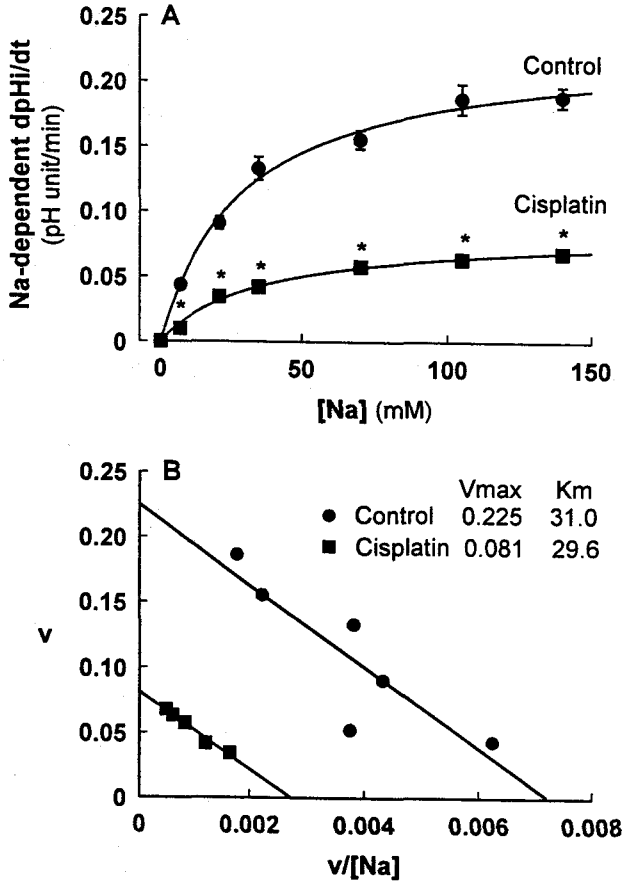

Fig. 5. A: Changes in the initial rate of Na^+-dependent pHi recovery (Na^+-dependent dpHi/dt) as a function of Na^+ concentration in the medium in control and cisplatin-treated OK cells. The Na^+-dependent dpHi/dt was obtained by subtracting the dpHi/dt in TMA-containing medium from that in Na^+-containing medium. Data represent mean±SE of 3 determinations. *Significantly different from the corresponding control value. B: Hofstee plots of the data in the panel A. Analysis of covariance indicates that the two regression lines are significantly different ($p<0.01$) in the intercept with ordinate (i.e., Vmax) but not in the slop (i.e., Km).

DISCUSSION

The data of the present study clearly indicate that cisplatin exposure induces a defect in Na^+/H^+ antiporter in OK cells. When cells were treated with 50 μM cisplatin for 1 h and then tested after 24 h, the Na^+-gradient dependent pHi recovery from intracellular acid loading appeared to be significantly retarded (Figs. 3 and 4). The Na^+-independent component of pHi-recovery and the viability of the cell were not significantly affected. The cellular Na^+ content before the onset of transport study was not different between the control and cisplatin group (Table 1), thus the imposed Na^+ gradient was identical. It is, therefore, apparent that the Na^+/H^+ antiport mechanism was impaired by cisplatin treatment. Kinetic analysis indicated that the Vmax, but not the Km, of Na^+-dependent pHi recovery was altered by cisplatin treatment (Fig. 5).

The mechanism by which cisplatin induced this change is not entirely clear. In the kinetic analysis of carrier-mediated transport, the Vmax is determined by two factors: (1) the capacity of carrier system itself and (2) the proportion of adsorbed molecules which dissociate in a forward direction in unit time (Neame & Richards, 1972). The former depends mainly on the number of carrier sites. The latter depends on the probability of a substrate molecule to dissociate from a carrier site in a given time and the rate of turnover of carrier across the membrane. Since in the present study the Km (i.e., substrate affinity) was not changed, it is unlikely that the carrier-substrate dissociation was altered. It is more likely that cisplatin reduced the number of carriers or their turnover rate. It is not certain, however, which one of these was actually affected by cisplatin. In this respect, it is important to mention that in our previous studies on LLC-PK_1 renal epithelial cell line, cisplatin exposure caused a decrease in phlorizin binding sites as well as Vmax of Na^+-hexose symport activity without affecting the Km (Lee et al, 1997), which indicates that the number of hexose transport carriers is reduced by cisplatin. Phelps et al (1987) have shown that cisplatin treatment induces partial loss of apical microvilli in the proximal tubule. If this happened in OK cells in the present study, the area of apical

membrane containing transporters would be reduced. Since, however, the Na^+-independent component of pHi recovery, which would be proportional to the membrane area for H^+ diffusion, was not decreased in cisplatin-treated cells (Figs. 3, 4), the apical membrane area may not be changed significantly. Thus, the drastic reduction in Vmax in cisplatin-treated cells, as observed in the present study, is most likely accounted for by a reduction in carrier density in the membrane. Since cisplatin treatment is known to inhibit macromolecule synthesis in renal tissues (Bodenner et al, 1986; Tay et al, 1988), it is speculated that synthesis of membrane transport carriers, such as Na^+/H^+ antiporter, is impaired by cisplatin treatment.

Lacchini et al (1992) have observed in renal tubular microperfusion studies in rats that cisplatin treatment resulted in an increase in proximal tubular acidification half time and a decrease in HCO_3^- reabsorption, without altering H^+ backflux, which suggest that proximal tubular H^+ secretion was impaired. Such a defect in H^+ secretion may be associated with a reduction in Na^+/H^+ antiport capacity, in light of the present study.

Field et al (1989) have used electron microprobe analysis to measure Na^+ concentrations in proximal tubules and found that Na^+ entry into the tubular cell was reduced by cisplatin. Since a major portion of proximal tubular Na^+ entry is mediated by the Na^+/H^+ antiporter, these data are compatible with impaired Na^+/H^+ antiporter in proximal tubular cells.

In conclusion, the present study have shown that cisplatin treatment could reduce the Na^+/H^+ antiport capacity, without altering substrate affinity, in OK renal epithelial cells. Such changes may be responsible for the impaired proximal tubular acidification and HCO_3^- reabsorption, as previously observed in intact animals.

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REFERENCES

Baldew GS, Boymans AP, Mol JGJ, Vermeulen NPE. The influence of ebselen on the toxicity of cisplatin in LLC-PK1 cells. *Biochem Pharmacol* 44: 382−387, 1992

Blachley JD, Hill JB. Renal and electrolyte disturbances associated with cisplatin. *Ann Intern Med* 95: 628−632, 1981

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248−254, 1976

Bodenner DL, Dendon PC, Jeng PC, Borch RF. Effect of diethyldithiocarmamate on cis-platinum-induced cytotoxicity, DNA cross-linking, and -glutamyl transpeptidase inhibition. *Cancer Res* 46: 2745−2750, 1986

Dagher G, Sauterey C. Regulation of intracellular pH in rabbit cortical connecting tubule and cortical collecting duct. *Biochim Biophys Acta* 902: 269−277, 1987

Daugaard G, Abildgaard U, Holstein-Rathlou N-H, Leyssac PP, Amtorp O, Dikhoff TC. Acute effect of cisplatin on renal hemodynamics and tubular function in dog kidneys. *Renal Physiol* 9: 308−316, 1986

Daugaard G, Abildgaard U, Larsen S, Holstein-Rathlou N-H, Amtorp O, Olsen HP, Leyssac PP. Functional and histological changes in dog kidneys after administration of cisplatin. *Renal Physiol* 10: 54−64, 1987

Daugaard G, Rossing N, Rorth M. Effects of high-dose cisplatin on glomerular function in the human kidney. *Cancer Chemother Pharmacol* 21: 163−167, 1988

Dentino M, Luft FC, Yum NM, Einhorn LH. Long term effect of cis-diamminedichloride platinum (CDDP) on renal function and structure in man. *Cancer* 41: 1274−1281, 1978

Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner NW. Mechanism of cisplatinum nephrotoxicity: II. Morphologic observations. *J Pharmacol Exp Ther* 7: 551−556, 1980

Field MJ, Bostrom TE, Seow F, Gyoery AZ, Cockayne DJH. Acute cisplatin nephrotoxicity in the rat. Evidence for impaired entry of sodium into proximal tubule cells. *Pflugers Arch* 414: 647−650, 1989

Gennari FJ, Kolb CH, Murer H. Influence of extracellular pH and perfusion rate on Na^+/H^+ exchange in cultured opossum kidney cells. *Pflugers Arch* 420: 153−158, 1992

Guggino WB, London R, Boulpaep EL, Giebisch G. Chloride transport across the basolateral cell membrane of the necturus proximal tubule: dependence on bicarbonate and sodium. *J Membr Biol* 71: 227−240, 1983

Helmle-Kolb C, Montrose MH, Murer H. Parathyroid hormone regulation of Na^+-H^+ exchange in opossum kidney cells: Polarity and mechanisms. *Eur J Physiol* 416: 615−623, 1990

Koyama H, Goodpasture C, Miller MM, Teplitz RL, Riggs AD. Establishment and characterization of a cell

line from the American opossum (Didelphys virginiana). *In Vitro* 14: 239−246, 1978

Lacchini MLS, Lopes AG, Malnic G, Giebisch G. Cisplatinum-induced lesion of proximal tubule acidification in the rat. *Renal Physiol Biochem* 15: 106−112, 1992

Lee SK, Kim JY, Yu TH, Kim KR, Kim KH, Park YS. Effect of cisplatin on sodium-dependent hexose transport in LLC-PK1 renal epithelial cells. *Korean J Physiol Pharmacol* 1: 35−43, 1997

Malmstrom K, Murer H. Parathyroid hormone inhibits phosphate transport in OK cells but not in LLC-PK_1 and JTC-12. P3 cells. *Am J Physiol* 251: C23−C31, 1986

Malmstrom K, Stange G, Murer H. Identification of proximal tubular transport functions in the established kidney cell line, OK. *Biochim Biophys Acta* 902: 269−277, 1987

Malnic G. Hydrogen secretion in renal cortical tubules: Kinetic aspects. *Kid Int* 32: 136−150, 1987

Mavichak V, Wong NLM, Quamme GA, Magil AB, Sutton RAL, Dirks, JH. Studies on pathogenesis of cisplatin-induced hypomagnesemia in rats. *Kid Int* 28: 914−921, 1985

Montrose MH, Murer H. Polarity and kinetics of Na^+-H^+ exchange in cultured opossum kidney cells. *Am J Physiol* 259: C121−C133, 1990a

Montrose MH, Murer H. Regulation of intracellular pH by cultured opossum kidney cells. *Am J Physiol* 259: C110−C120, 1990b

Montrose MH, Thomas F, Murer H. Measurements of intracellular pH in single LLC-PK1 cells. *J Membrane Biol* 97: 63−78, 1987

Murer H, Krapf R, Kolb CH. Regulation of renal proximal tubular Na/H-exchange: A tissue culture approach. *Kid Int* 45: S23−S31, 1994

Neame KD, Richards TG. Elementary Kinetics of Membrane Carrier Transport. Wiley, New York, 1972

Phelps JS, Gandolfi AJ, Brendel K, Door RT. Cisplatin nephrotoxicity: in vitro studies with precision-cut rabbit renal cortical slices. *Toxicol Appl Pharmacol* 90: 501−512, 1987

Quamme G, Biber J, Murer H. Sodium-phosphate cotransport in OK cells: inhibition by PTH and adaptation to low phosphate. *Am J Physiol* 257: F967−F973, 1989

Roseneweig M, Von Hoff DD, Slavik M, Muggia FM. Cis-Diamminedichloroplatinum (II), a new anticancer drug. *Ann Intern Med* 86: 803−812, 1977

Ross BD. Clinical Physiology of Acid-Base and Electrolyte Disorders. 3rd ed. McGraw-Hill, New York, 1989

Safirstein R, Miller P, Dikman S, Lyman N, Shapiro C. Cisplatin nephrotoxicity in rats: Defect in papillary hypertonicity. *Am J Physiol* 241: F175−F185, 1982

Safirstein R, Winston J, Goldstein M, Moel D, Dikman S, Guttenplan J. Cisplatin nephrotoxicity. *Am J Kid Dis* 8: 356−367, 1986

Schilsky RL, Anderson T. Hypomagnesemia and renal magnesium wasting in patients receiving cisplatin. *Ann Intern Med* 90: 929−931, 1979

Tay LK, Bregman CL, Masters BA, Williams PD. Effects of cis-diaminedichloroplatinum (II) on rabbit renal proximal tubule cells in culture. *Cancer Res* 48: 2538−2543, 1988

Van den Bosch L, De Smedt H, Borghgraef. Characterization of Na^+-dependent hexose transport in OK, an established renal epithelial cell line. *Biochim Biophys Acta* 979: 91−98, 1989

Winston JA, Safirstein R. Reduced renal blood flow in early cisplatin-induced acute renal failure in the rat. *Am J Physiol* 249: F490−F496, 1985