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Effect of Cisplatin on Sodium-Dependent Hexose Transport in LLC-PK$_1$ Renal Epithelial Cells

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Cis-dichlorodiammine platinum II (Cisplatin), an effective chemotherapeutic agent, induces acute renal failure by unknown mechanisms. To investigate direct toxic effects of cisplatin on the renal proximal tubular transport system, LLC-PK$_1$ cell line was selected as a cell model and the sugar transport activity was evaluated during a course of cisplatin treatment. Cells grown to confluence were treated with cisplatin for 60 min, washed, and then incubated for up to 5 days. At appropriate intervals, cells were tested for sugar transport activity using α-methyl-D-[^{14}C]glucopyranoside (AMG) as a model substrate. In cells treated with 100 μM cisplatin, the AMG uptake was progressively impaired after 3 days. The viability of cells was not substantially changed with cisplatin of less than 100 μM, but it decreased markedly with 150 and 200 μM. In cisplatin-treated cells, the Na^+-dependent AMG uptake was drastically inhibited with no change in the Na^+-independent uptake. Kinetic analysis indicated that Vmax was suppressed, but Km was not altered. The Na^+-dependent phlorizin binding was also decreased in cisplatin-treated cells. However, the AMG efflux from preloaded cells was not apparently retarded by cisplatin treatment. These data indicate that the cisplatin treatment impairs Na^+-hexose cotransporters in LLC-PK$_1$ cells and suggest strongly that defects in transporter function at the luminal plasma membrane of the proximal tubular cells constitute an important pathogenic mechanism of cisplatin nephrotoxicity.

Key Words: cisplatin, LLC-PK$_1$ cell, Na-hexose cotransport, phlorizin

INTRODUCTION

Cisplatin (cis-dichlorodiammine platinum II), an inorganic platinum compound, is an extremely effective anticancer agent for several types of cancer including testicular, bladder, ovarian, osteogenic, head and neck, and uterine cervical carcinomas (Einhorn & Williams, 1979). These tumors are treated most effectively by using relatively high dose of regimens of chemotherapeutic agents. Unfortunately, adverse effects of cisplatin increase with increased dosage and these adverse effects are dose-limiting (Daugaard & Abildgaard, 1989).

Cisplatin has been shown to induce nephrotoxicity (Safirstein et al, 1987), ototoxicity (Komune et al, 1981), myelosuppression (Gringieri et al, 1988), gastrointestinal toxicity, nausea, peripheral neuropathy, and anaphylactic reaction (Von Hoff et al, 1979), the most severe of these in humans being nephrotoxicity (Borch, 1993).

The vulnerability of the kidney to cisplatin is linked to its primary role in the metabolism of the drug. It is responsible for most of the platinum excretion and is a major site for accumulation as well as for retention (Borch, 1993). Autoradiographic studies in rats (Safirstein et al, 1987) have shown that intravenously injected [^{195m}Pt]cisplatin is largely accumulated into the juxtamedullary and outer stripe region of the kidney, especially at the S3 segment (*pars recta*) of the proximal nephron. The S3 segment is known to be the principal site of morphological lesion by cisplatin in rats (Dobyan et al, 1980; Safirstein et al, 1981). In rabbits, acute necrosis was observed to be more pronounced in the proximal convoluted tubules (Tay et al, 1988). In humans,

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major damages have been observed in distal part of proximal tubule or in more distal nephron segment (Dentino et al, 1978).

Studies in dogs and rats (Daugaard et al, 1986; 1988) indicated that acute proximal tubular dysfunction is the onset of renal toxicity of cisplatin. Following the cisplatin administration renal blood flow and glomerular filtration rate are not altered, but proximal tubular sodium and fluid reabsorptions are drastically diminished. At 3 days after cisplatin administration, renal blood flow and glomerular filtration rate are also markedly decreased, probably due to increased vascular resistance and decreased filtration pressure (Winston & Safirstein, 1985). At this stage, despite the reduction in filtered load, impaired sodium transport in the thick ascending limb of Henle's loop and further deterioration of proximal and distal tubular sodium reabsorption result in polyuria (Seguro et al, 1989). In patients undergoing treatment with cisplatin, urinary excretion of β-2-microglobulin has been found to increase (Cohen et al, 1981; Daugaard & Abildgaard, 1989). Since this substance is normally filtered freely across the glomerular membrane and nearly completely reabsorbed by the proximal tubule (Bumah et al, 1982), an increase in its renal excretion indicates that proximal tubular transport function is impaired. Likewise, urinary excretions of alanine aminopeptidase, leucine aminopeptidase and N-acetyl-β-D-glucosaminidase, the enzymes preferentially located in the proximal tubular cells, increase consistently in patients receiving high doses of cisplatin (Daugaard & Abildgaard, 1989).

These results indicate that the most prominent feature of the cisplatin-induced nephrotoxicity is the proximal tubular dysfunction. Since the proximal tubule reabsorbs most of the filtered solutes along with sodium, it is entirely possible that various sodium-coupled transport processes are affected by cisplatin treatment. The present study was, therefore, undertaken to examine this possibility. LLC-PK_1 cell line was selected as a model system to evaluate direct cellular effects of cisplatin on sodium-coupled sugar transport. The LLC-PK_1 cell is a continuous cell line derived from the kidney of a Hampshire pig (Hull et al, 1976). When grown to confluence, these cells express many characteristics of proximal tubular epithelia, including sodium-dependent hexose transporter similar to that in *pars recta* (Moran et al, 1982; Handler, 1986), and thus have been extensively used to study proximal tubular injury by xenobiotics (Schwertz et al, 1986; Inui et al, 1988; Montine & Borch, 1988; Chan et al, 1989).

MATERIALS AND METHODS

Cell culture

LLC-PK_1 cell line was obtained from the American Type Culture Collection (ATCC) and maintained by serial passages in plastic culture flasks. All experiments were performed on passages 200-220. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 1% antibiotic/antimycotic solution (Gibco) in an atmosphere of 5% CO_2-95% air at 37°C. The culture was fed with fresh medium twice a week. When cell growth reached saturation density, subcultures were prepared by treatment with 0.05% trypsin-0.02% EDTA for 10-15 min at 37°C and the cells were suspended by gentle shaking of the culture flasks. The cells were plated at 1:6 dilution in 250 ml plastic culture flasks (growth area: 75 cm^2). The logarithmic phase of cell growth was shown between 24 and 48 h with a doubling time of approximately 14 h. Confluence occurred after 3-4 days in culture. The cell monolayers were maintained in serum-free medium for 2 days before drug treatment. Only monolayers in which dome formation occurred were used for the experiments.

Cytotoxicity of 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 (pH 7.4), saturated with 5% CO_2-95% air at 37°C. Monolayers of LLC-PK_1 cells were washed twice with phosphate-buffered saline (PBS) and then exposed to the cisplatin-containing medium for 60 min. Upon completion of exposure the monolayers were washed three times with PBS to remove residual drug, and subsequently were incubated in the DMEM. In control experiments, monolayers were exposed to the vehicle (plain salt medium).

To determine the cell viability, monolayers of LLC-PK_1 cells were detached with trypsin. 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. The protein concentration was deter-

mined according to Bradford (1976), using the Bio-Rad Protein Assay Kit with bovine γ-globulin as a standard.

Measurement of hexose transport

The ability of LLC-PK$_1$ cells to transport hexose was determined in Hank's salt solution (HSS) using α-methylglucopyranoside (AMG) as a model substrate. AMG is a nonmetabolized glucose analogue known to share Na^+-dependent apical membrane pathway in the mammalian proximal tubule (Silverman, 1976) and in LLC-PK$_1$ epithelia (Rabito, 1981; Amsler & Cook, 1982). The HSS consisted 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, 10 HEPES, and 5.5 glucose (pH 7.2). In Na^+-free HSS NaCl was replaced by equimolar concentration of choline chloride, and $NaHCO_3$ and Na_2HPO_4 were replaced by respective K^+-salts. After removing culture medium, each culture flask was washed twice with PBS and then monolayers were trypsinized. The cell suspension was centrifuged at 1,100 rpm for 5 min and then suspended in PBS, followed by centrifugation at 1,100 rpm for 5 min. The cells were then washed with Na^+-free HSS and centrifuged again. The packed cell pellet was resuspended in Na^+-free HSS and the cell suspension was preincubated for 30 min at 37°C.

Transport of AMG into LLC-PK$_1$ cells was determined using a rapid filtration method. An aliquot of cell suspensions was incubated in 9 volumes of incubation medium (HSS) containing AMG (100 μM in most cases) and ^{14}C-AMG (1 μCi/ml) at 25°C. At appropriate intervals, a 100 μl aliquot was removed and quickly filtered through Millipore filter (type HA, pore size 0.45 μm) which was soaked overnight in distilled water prior to use. The filter was washed with 5 ml of ice-cold stop solution. ^{14}C-AMG in the filter was dissolved into 6 ml of Lumagel (Lumac, AC Landggaf, the Netherlands) and the ^{14}C activity was counted on a liquid scintillation counter (Packard Tricarb 4530C). The AMG uptake by the cell was expressed as pmoles per mg protein for a given time.

In AMG efflux experiments, cells were first loaded with AMG by incubating them in a medium containing ^{14}C-AMG (10 μCi/ml) for 2 h at 25°C. The incubation mixture was then diluted (1/20) with a ^{14}C-AMG-free medium containing phlorizin (100 μM). At appropriate intervals, incubations were terminated and the ^{14}C-AMG retained by the cells was determined.

In some experiments phlorizin binding to LLC-PK$_1$ cells was determined using a rapid filtration method. An aliquot of cell suspensions was incubated in 9 volumes of incubation medium (HSS) containing 135 nM phlorizin/5 μCi ^{3}H-phlorizin at 25°C for 30 min. In preliminary experiments, we have observed that binding reached a steady state after 10 min. At the end of the incubation period, a 100 μl aliquot was removed and filtered through Millipore filter (type HA, pore size 0.45 μm). Radioactivity retained in the filter was determined as described above.

Chemicals and materials

Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, trypsin, antibacterial/antimycotic solution were purchased from Gibco (Grand Island, NY, USA). α-methyl-D-[^{14}C]glucopyranoside and ^{3}H-phlorizin were obtained from New England Nuclear (Boston, MA, USA). Cisplatin, -methyl-D- glucopyranoside, phlorizin, and ouabain were from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were of analytical grade.

Statistical analysis

Statistical evaluation of data was done using Students t-test. Differences with $p<0.05$ were considered statistically significant.

RESULTS

Uptake of α-methylglucopyranoside (AMG)

Previous studies on the LLC-PK$_1$ cells (Moran et al, 1983; 1984) have shown that the capacity of the cell to concentrate AMG increases progressively during culture. In order to investigate if the transport activity continues to change even after confluence, we measured the rate of AMG uptake as a function of time after confluence. The results depicted in Fig. 1 (solid line) indicated that the AMG uptake rate increased rapidly (by ~ 5 folds) during the first 2 days after confluence and slowly thereafter, reaching a maximum after 7 days. The protein content of the culture plate did not undergo significant variation (data not shown), reflecting no net increase in the cell number.

Fig. 1 also depicts changes in AMG transport activity of cisplatin-treated cells (dashed line). Two

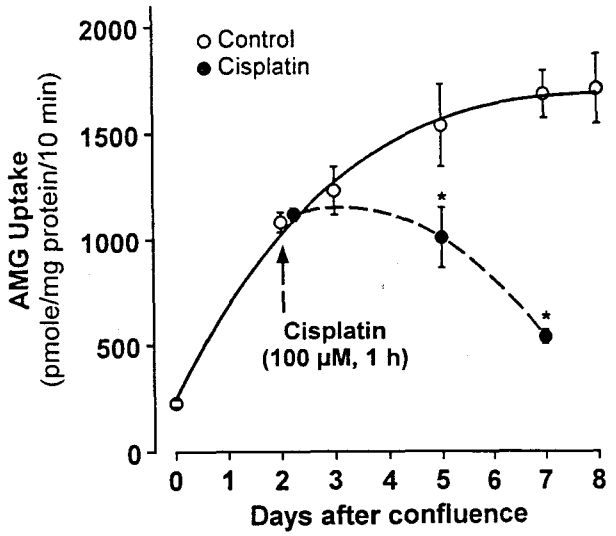

Fig. 1. Changes in α-methylglucopyranoside (AMG) uptake by LLC-PK$_1$ cells after confluence. In control group, cells grown to confluence were maintained in serum-free culture medium until tested for AMG uptake at the times indicated. In cisplatin group, 2 days after confluence cell monolayers were exposed to 100 μM cisplatin for 60 min, washed and maintained in normal serum-free culture medium. At the time of the test, cells were first preincubated in Na^+-free (choline) medium for 30 min at 37°C to normalize intracellular Na^+ concentration, and then incubated in the Hanks salt solution (HSS) containing 145 mM Na^+ and 100 μM AMG (with 1 μCi/ml ^{14}C-AMG) for 10 min at 25°C. Data represent mean ± SE of 4-6 determinations. * significantly different ($p < 0.05$) from the respective control value.

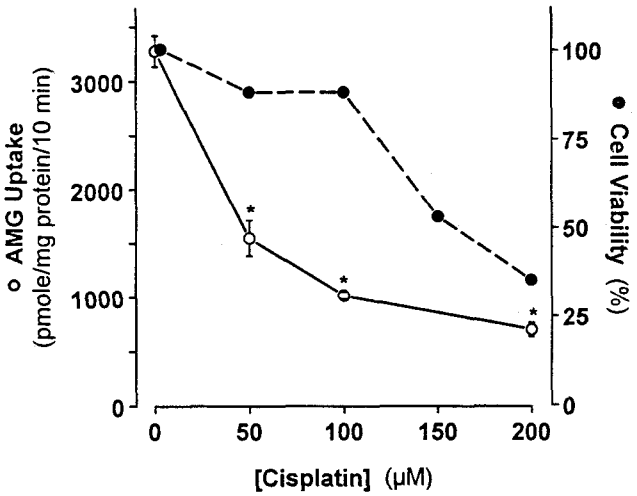

Fig. 2. Effects of cisplatin treatment on LLC-PK$_1$ cell viability and AMG uptake as a function of cisplatin concentration. Cells grown to confluence were exposed to cisplatin at the concentrations indicated for 60 min, washed, and incubated with fresh medium for 5 days before the test. Cell viability was evaluated by trypan blue exclusion and quantified by measuring the amount of protein remaining attached to the culture plate (2.5 cm2). For AMG uptake, cells were first preincubated in Na^+-free medium for 30 min at 37°C, and incubated in the HSS containing 145 mM Na^+ and 100 μM AMG for 10 min at 25°C. Data represent the mean of 2 determinations for viability and mean ± SE of 4 determinations for AMG uptake. * significantly different ($p < 0.05$) from the respective control (0 μM cisplatin) value.

days after confluence cell monolayers were exposed to 100 μM cisplatin for 1 h, washed, and then incubated in serum-free medium for up to 5 days. Over the first 6 h, following cisplatin exposure, no significant impairment of AMG uptake appeared to develop. However, the uptake decreased significantly 3 days after and further 5 days after the drug exposure, as compared with the matched control. On the basis of these results, we analyzed cisplatin effect on AMG transport 5 days after exposure in all subsequent experiments.

Fig. 2 (solid line) illustrates dose dependence of AMG uptake by LLC-PK$_1$ cells to cisplatin exposure. Cells grown to confluence were exposed to cisplatin at 0, 50, 100, or 200 μM for 60 min, washed and incubated with fresh medium for 5 days before the test. The uptake decreased progressively as the cisplatin concentration increased. The value for the cells exposed to 50 μM, 100 μM, and 200 μM cisplatin was approximately 47%, 30% and 20% of the control, respectively. The viability of cell decreased slightly (by ~ 12%) at 50 and 100 μM, but substantially (by ~ 65 %) at 200 μM cisplatin (Fig. 2 dashed line). On the basis of these results, we chose 100 μM as the concentration of cisplatin in subsequent experiments.

Fig. 3 depicts the effect of cisplatin exposure on the Na^+-dependent and Na^+-independent AMG uptakes by LLC-PK$_1$ cells. Control and cisplatin (100 μM)-treated cells were incubated in Na^+-containing or Na^+-free (choline) medium and AMG uptake was determined for various time periods. In the control group, the AMG uptake in the Na^+-containing medium increased rapidly during the initial 20 min and slowly thereafter. The AMG uptake in Na^+-free medium, was very much less than that in Na^+-containing medium. In cisplatin-exposed cells, the AMG

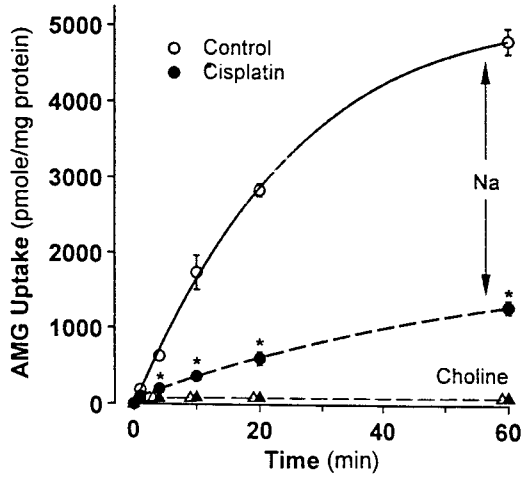

Fig. 3. Time courses of Na^+-dependent and Na^+-independent AMG uptake. Cells grown to confluence were exposed to normal or cisplatin (100 μM)-containing medium for 60 min, washed, and incubated for 5 days before the test. For AMG uptake, cells were first preincubated in Na^+-free medium for 30 min at 37°C, and then incubated in the HSS containing 145 mM Na^+ (or choline) and 100 μM AMG for the times indicated at 25°C. Data represent mean ± SE of 6 determinations. * significantly different ($p<0.05$) from the respective control value.

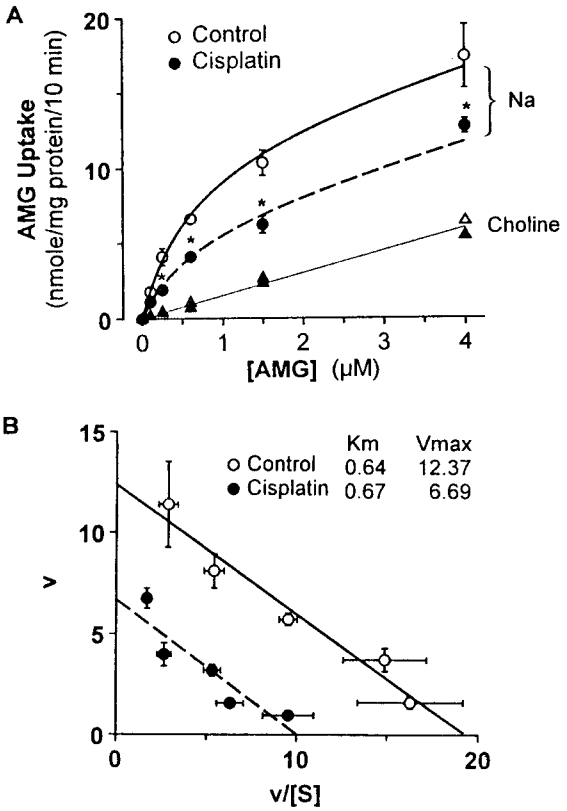

Fig. 4 A. Initial (10 min) rate of AMG uptake by control and cisplatin-treated LLC-PK_1 cells as a function of substrate concentration. Cells grown to confluence were exposed to normal or cisplatin (100 μM)-containing medium for 60 min, washed and incubated with fresh medium for 5 day before the test. Cells were preincubated in Na^+-free medium for 30 min at 37°C, and the AMG uptake was determined for 10 min at 25°C both in 145 mM Na^+ containing and Na^+-lacking HSS, and the difference was taken as the . Na^+-dependent uptake. Data represent mean ± SE of 4 determinations. * significantly different ($p<0.05$) from the respective control value.
B. Hofstee plot of Na^+-dependent AMG uptake. The Na^+-dependent uptake was calculated by subtracting the uptake in the absence of Na^+ from that in the presence of Na^+ in each substrate concentration. Data based on Fig 4 A.

uptake in Na^+-free medium was comparable to that of control group, but that in Na^+-containing medium was markedly suppressed. The initial rate of Na^+-dependent transport (i.e., the difference in uptake between Na^+-containing- and Na^+-free media) calculated for the first 10 min was on the average 170 and 25 pmoles/mg protein/min in the control and cisplatin-exposed cells, respectively.

Fig. 4A presents kinetics of AMG uptake. The AMG uptake in Na^+-free (choline) medium increased linearly with the AMG concentration, with identical rate in the control and cisplatin group. On the other hand, the uptake in Na^+-containing medium increased curvilinearly showing a clear tendency for saturation and was markedly depressed in cisplatin-treated cells. Hofstee analysis of the Na^+-dependent component of AMG uptake (Fig. 4B) gave an apparent Km of 0.64 ±0.08 mM for the control and 0.67±0.17 for the cisplatin group and the Vmax of 12.37±0.92 n mole/mg protein/10 min for the control and 6.69± 1.08 for the cisplatin group. It thus appeared that the cisplatin treatment resulted in a marked depression of Vmax with no significant alterations in Km. Such results would indicate that the capacity for Na^+-hexose cotransport was reduced by cisplatin treatment. The value of Km observed in this study was comparable to that reported by others in LLC-PK_1 cells (0.75 mM, Rabito, 1981).

Phlorizin binding

Previous studies on LLC-PK_1 cells have shown that

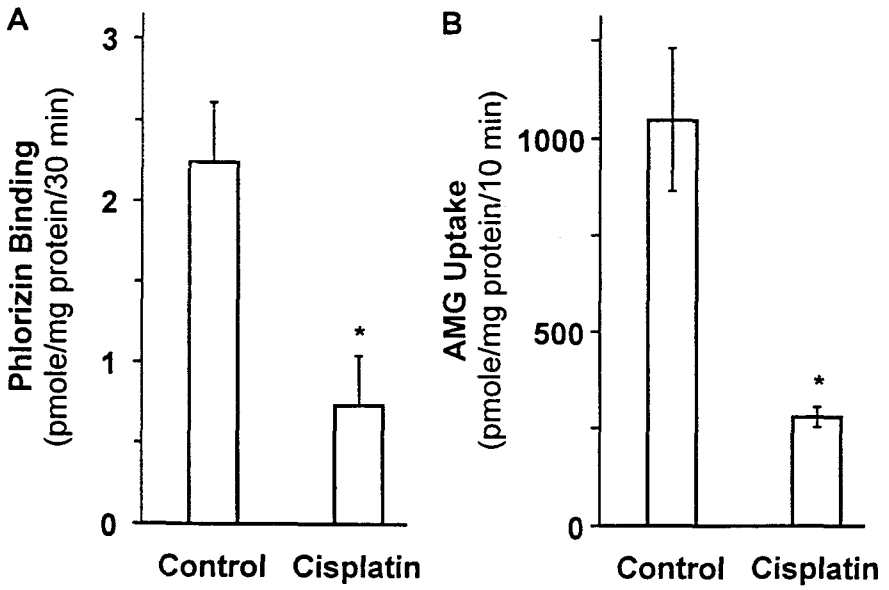

Fig. 5. Na^+-dependent phlorizin binding and AMG uptake by control and cisplatin-treated LLC-PK_1 cells. Cells grown to confluence were exposed to normal or cisplatin (100 μM)-containing medium for 60 min, washed and incubated for 5 days before the test. Cells were preincubated in Na^+-free medium for 30 min at 37°C and incubated in the HSS containing 145 mM Na^+ (or choline) and 100 μM substrate (phlorizin/1 μCi/ml ^{3}H-phlorizin or AMG/1 μCi/ml ^{14}C-AMG) for 30 min (phlorizin binding) or 10 min (AMG uptake) at 25°C. The Na^+-dependent component was obtained by subtracting the value in choline-containing medium from that in Na^+-containing medium. Data represent mean ± SE of 4 determinations. * significantly different ($p<0.05$) from the respective control value.

there exist a linear correlation between the capacities of Na^+-dependent hexose uptake and Na^+-dependent phlorizin binding under a variety of conditions (Moran et al, 1983; Amsler & Cook, 1985). We therefore attempted to directly evaluate changes in the number of Na^+-hexose cotransporters in cisplatin-treated cells by measuring phlorizin binding.

Fig. 5A shows that the amount of steady-state Na^+-dependent phlorizin binding was markedly reduced in cisplatin (100 μM)-treated than in control cells. The value for cisplatin group (0.73 p mole/mg protein) was approximately 30 % of the control value. In parallel run, a similar reduction of Na^+-dependent AMG uptake rate was observed in cisplatin-treated cells (Fig. 5B). These results indicate that the total number of functional carriers in the cell was reduced by cisplatin treatment.

Efflux of α-methylglucopyranoside

To further elucidate the mechanism of cisplatin-induced reduction of hexose transport capacity, we next evaluated the rate of AMG efflux from preloaded LLC-PK_1 cells. In these experiments, efflux via the apical Na^+-hexose cotransporter was prevented by phlorizin (100 μM). Since AMG is not apparently transported by basolateral hexose carrier in LLC-PK_1 cells (Rabito, 1981; Amsler & Cook, 1982) these experiments measure AMG efflux via passive diffusion across the apical and basolateral membranes (Moran et al, 1983). Fig. 6 depicts natural logarithms of the relative amount of AMG remaining in the cell at the end of each efflux period. The efflux rate constant (ke) calculated from the slope between 15 and 45 min was not significantly different between the control (0.00755/min) and cisplatin-treated cells (0.00767/min), suggesting that the area for AMG diffusion was not substantially changed by cisplatin treatment.

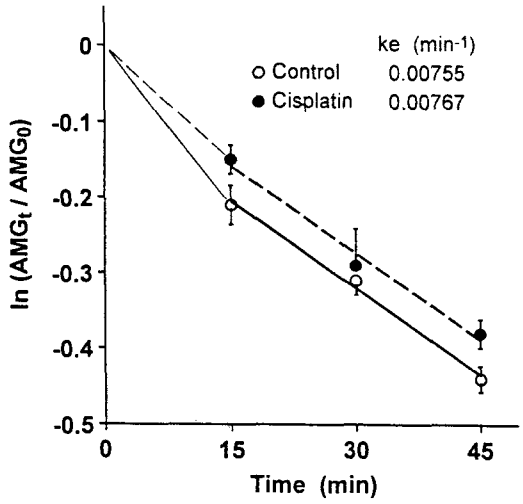

Fig. 6. Efflux of AMG from control and cisplatin-treated LLC-PK$_1$ cells. Cells grown to confluence were exposed to normal or cisplatin (100 μM)-containing medium for 60 min, washed, and incubated for 5 days before the test. For AMG efflux, cells were first loaded with ^{14}C-AMG by incubating them in the HSS containing ^{14}C-AMG (10 μCi/ml) for 2 h at 25°C. Loaded cells were then diluted (1/20) with a ^{14}C-free medium containing 100 μM phlorizin at time zero. Natural logarithms of the relative amount of AMG remaining in the cell at the end of each efflux time was plotted against time. Data represent mean ±SE of 5 determinations.

DISCUSSION

Studies on experimental animals have shown that cisplatin-induced nephrotoxicity is initiated by a defect in proximal tubular Na^+ transport (Daugaard & Abildgaard, 1989). The cellular mechanism responsible for this change has not been clearly identified. One theory to explain cisplatin nephrotoxicity is related to an interaction between cisplatin and Na^+-K^+-ATPase. Phelps et al (1987) observed a rapid decline of ATP and K^+ content in renal cortical slices treated with cisplatin, which may support this hypothesis. Cisplatin inhibition of Na^+-K^+-ATPase activity *in vitro* has been reported in renal tissue homogenates of rats (Daley-Yates & McBrien, 1982) and humans (Nechay & Neldon, 1984). In these studies, however, exceedingly high concentrations of cisplatin was required to achieve a significant enzyme inhibition. More recently, Uozumi and Litterst (1985) and Tay et al (1988) have observed that concentrations of cisplatin lower than 100 μM could not induce a significant inhibition of ATPase, unless the enzyme was exposed for a prolonged period. They, therefore, concluded that the ATPase inhibition may not be a critical course of cisplatin nephrotoxicity. Another theory for cisplatin nephrotoxicity involves unoupling of oxidative phosphorylation in mitochondria (Gordon & Gattone, 1986; Brady et al, 1990). A number of studies, however, have pointed out that mitochondria, as well as ATPase, may not be important, early pathogenic targets for cisplatin (Uozumi & Litterst, 1985; Safirstein et al, 1987; Tay et al, 1988).

If these were the case, then the early impairment of proximal tubular Na^+ transport by cisplatin may not be accounted for by an alteration of active transport step at the basolateral membrane, but is associated with an inhibition of Na^+ entry across the apical (brush border) membrane. In fact, studies of Field et al (1989) in rats have implied that Na^+ entry into proximal tubule cells is attenuated in acute cisplatin nephrotoxicity. Whether this impairment affects Na^+-coupled solute transport in apical membrane has not been identified.

The present study clearly demonstrated that the Na^+-dependent hexose transport in LLC-PK$_1$ cells is inhibited by cisplatin treatment. In cells exposed to 100 μM cisplatin, AMG uptake in the presence of extracellular Na^+ appeared to be markedly suppressed with no change in Na^+-independent uptake (Fig. 3). This impairment of the transport capacity became apparent 3 days after the drug exposure (Fig. 1). Such a delay in drug effect is consistent with the time course of nephrotoxicity observed in cisplatin-treated animals and patients (Safirstein et al, 1987).

In the kinetic analysis of Na^+-dependent AMG uptake, Vmax, but not Km, was markedly reduced in cisplatin-treated cells (Fig. 4). This implies that the cisplatin treatment resulted in a decrease of Na^+-hexose cotransport carriers. As in renal proximal tubule, the Na^+-hexose cotransport carriers in LLC-PK$_1$ cells are localized in the apical plasma membrane (Rabito, 1981; Moran et al, 1983) and can be titrated by specific phlorizin binding (Rabito, 1981; Moran et al, 1983; Amsler & Cook, 1985). In the present study, the Na^+-dependent AMG uptake and the Na^+-dependent phlorizin binding determined in the same batches of cultures showed a parallel reduction in cisplatin-treated cells (Fig. 5). It is therefore apparent that the number of cotransport carriers in the cell was reduced by cisplatin treatment.

The mechanism with which cisplatin diminished the Na^+-hexose cotransporters is not certain at present. Morphological investigations have shown that cisplatin treatment induces partial loss of microvilli in the proximal tubule (Phelps et al, 1987). If this happened in LLC-PK_1 cells in the present study, the area of apical membrane containing Na^+-hexose cotransporters would be reduced. However, the AMG efflux study implied that this may not be the case. The AMG efflux was not significantly decreased in cisplatin-treated cells (Fig. 6). Since in these experiments the carrier-mediated efflux was prevented by phlorizin, the rate of efflux would be proportional to the membrane area for simple diffusion. The more likely reason for the impaired AMG uptake in cisplatin-treated cells is, therefore, a reduction of transporter density in the membrane. Several studies (Bodenner et al, 1986; Tay et al, 1988) have indicated that macromolecule synthesis in renal tissues is inhibited during an early period of cisplatin post-exposure. It is, therefore, possible that the synthesis of Na^+-hexose cotransport carrier protein is impaired by cisplatin exposure. Molecular biological studies of Na^+-AMG cotransporters would resolve this problem.

In the present study, the Na^+-pump activity at the basolateral membrane was not assessed. Since, however, cells were preincubated in Na^+-free medium before uptake study to normalize the amount of intracellular Na^+ content, the imposed Na^+ gradient may not be significantly different between the control and cisplatin-treated cells. Thus, the inhibition of AMG initial uptake observed in cisplatin treated cells may not be attributed to changes in Na^+- pump.

In summary, the present study clearly established that cisplatin exposure impairs the Na^+-sugar cotransport system in LLC-PK_1 cells. Since the Na^+-sugar cotransport system is one of the most representatives of the large number of functional markers associated the apical membrane of renal epithelial cells, the present results emphasizes that defect in transport functions at the proximal tubular luminal membrane is an important course of pathogenesis of cisplatin nephrotoxicity.

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