ISSN 1009-3095 Journal of Zhejiang University(SCIENCE) V.1,No.1,P.91-96,Jan.-Mar.,2000
http://www.zju.edu.cn; http://www.chinainfo.gov.cn/periodical

MITOCHONDRIAL DYSFUNCTION AT THE EARLY STAGE OF CISPLATIN-INDUCED ACUTE RENAL FAILURE IN RATS

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Received Dec.12, 1998; revision accepted Apr.15, 1999

Abstract: The present study was undertaken to clarify the pathogenesis of cisplatin-induced acute renal failure at the early stage. Male Sprague-Dawley rats were given an intravenous administration of 10 mg/kg cisplatin. 0.9% saline was infused into them at a rate of 2 ml/h for 3 h, starting with a 2-ml bolus injection before cisplatin administration. 3 h following cisplatin administration, no evident morphological abnormalities were found by both light and electron microscopy; there were also no significant changes in GFR. Thirty min after cisplatin injection, urine sodium and potassium excretion increased by 56% and 260% those of the control animals, respectively. Apparent renal mitochondrial respiration dysfunction was observed in cisplatin-treated rats 3 h later; the state 4 respiration increased by 100% and state 3 respiration, respiratory control ratio and carbonyl cyanide p-trifluoromethoxyphenyl hydrazone-uncoupled respiration decreased by 46%, 74% and 47% of the controls, respectively. The present data suggest that mitochondrial dysfunction may be a very early event in cisplatin-induced acute renal failure in rats.

Key words: acute renal failure, cisplatin, pathogenesis, kidney tubule, mitochondria
Document code: A **CLC number:** R69

INTRODUCTION

Cis-diamminedichloroplatinum(Ⅱ)(Cisplatin)is a widely used antineoplastic agent that has nephrotoxicity as a major dose-limiting side effect. The most common form of cisplatin-induced renal toxicity is non-oliguric acute renal failure. The underlying mechanism of this renal damage is still not well known. Offerman et al. reported that renal blood flow(RBF) was reduced prior to any change in glomerular filtration rate (GFR) in patients treated with cisplatin and proposed that reduction in RBF is an essential factor in initiating this nephrotoxicity (Offerman et al., 1984). However, no significant alterations of RBF and GFR were observed in dogs 0-4 h after cisplatin administration. Further studies showed that a significant reduction in absolute proximal reabsorption rates occurred both in rats and dogs immediately after administration of cisplatin while GFR and RBF remained essentially unchanged, suggesting that cisplatin-induced acute renal failure was initiated by proximal tubular impairment (Dauqaard 1990; Jones et al., 1985). Morphological studies of rats showed that cisplatin-induced lesion was located on the straight portion of the proximal tubules (S_3 Segment) (Jones et al., 1985; Chopra et al., 1982). But the early cellular events in renal toxicity from cisplatin are not fully elucidated.

The present study was aimed to clarify the pathogenesis of cisplatin-induced acute renal failure at the early stage, especially the early cellular events.

METHODS

1. *In vivo* experiments

Animal preparation

Male Sprague-Dawley rats weighing 250-350g were used for all experiments. They were anesthetized with sodium pentobarbital (60mg/kg, intraperitoneally) and then placed on a warmed operating table to maintain body temperature at about 37℃ throughout the experiment. The femoral vein was cannulated with a two-channel vein catheter for infusion of cisplatin

(CP) and fluid. Following a 2 ml bolus infusion, 0.9% saline was then infused into the femoral vein at a rate of 2 ml/h using a constant-infusion syringe pump.

After a midline laparotomy, the abdominal aorta below the renal arteries was catheterized with an intravenous catheter placement set and 0.1ml heparin was infused into the connecting tube to avoid blood coagulation. Mean arterial blood pressure (MABP) was measured using a strain-gauge transducer(Gould, Statham) and a magnifier (Hugo Sachs Elektronik, March-Hugstetten) connected to a recorder (ABB Goerz-Metrawatt, Austria). During the experiment, MABP of all animals ranged from 13.3 kPa to 16 kPa.

A plastic tube was inserted into the bladder. Urine was collected in plastic test tube every 30-min for up to 3 h following cisplatin injection, and used for calculation of urine volume, determination of creatinine and sodium or potassium concentrations. At the termination of the 3 h *in vivo* experiment, 2 ml blood sample was taken from the portal vein, placed at 4 ℃ for 30 min and centrifuged at 735(× g) for 10 min to separate serum for creatinine determination. Meanwhile, the superior mesenteric artery and the aorta above and below the renal arteries were ligated and the kidneys were irrigated via the aortal catheter with a perfusion medium (in mmol/L: 138 NaCl, 4.8 KCl, 1 $CaCl_2 \cdot 2H_2O$, 1 $MgCl_2 \cdot 6H_2O$, 8.25 TRIS, 0.8 $Na_2HPO_4 \cdot 2H_2O$, 0.27 KH_2PO_4 and 50 mannitol buffered at pH 7.35 with HCI mol/L). Either kidney was taken immediately for isolation of mitochondria. In addition, the pedicles of those kidneys used for morphological studies were tied before perfusion.

Experimental groups

The animals were divided into 3 groups at random. Control group($n = 21$): under anesthesia, either kidney was removed immediately after a midline laparotomy and renal perfusion, used for mitochondrial isolation. Sham-operated (SHAM-OP) group ($n = 16$): only 0.9% saline was infused. CP group($n = 12$): 10 mg/kg cisplatin was injected over several minutes after a 2-ml bolus infusion of 0.9% saline.

2. *In vitro* experiments

Mitochondrial isolation

Mitochondria of the kidney were isolated according to the method of Goldstein (Goldstein, 1975). Mitochondrial pellets were resuspended in incubation medium (in mmol/L: 290 saccharose, 2 HEPES, 2.5 K_2HPO_4, 2.5$MgCl_2 \cdot 6H_2O$, 0.5 EDTA, 5 malate, 5 glutamate and 0.1% bovine serum albumin at pH 7.4 with KOH mol/L) with protein concentration at about 2.14 mg/ml.

Measurement of mitochondrial respiration

Mitochondrial oxygen consumption was measured polarographically at 25 ℃ in a closed 0.45 ml reaction chamber fitted with a Clark O_2 electrode (Eschweiler, Kiel), connected to a recorder. Before starting measurements, the apparatus was calibrated with a calibration medium (2% Na_2SO_3, 0.2% $Na_2B_4O_7$).

Mitochondrial suspension (0.45 ml) containing 5 mmol/L malate and 5 mmol/L glutamate was added to the chamber to initiate the study of mitochondrial respiration. State 3 respiration was induced by the addition of 0.5 mmol/L ADP. After the ADP effect had worn off (phosphorylated to ATP), state 4 respiration was measured. Respiratory control ratio (RCR) is the quotient of state 3/state 4 respiration used as an indicator of the integrity of the mitochondrial preparation, with high values representing a tight coupling between ADP phosphorylation to ATP and electron transport. In addition, the uncoupled rate of O_2 consumption was measured after the addition of 2 nmol/L carbonyl cyanide p-trifluoromethoxyphenyl hydrazone (FCCP) providing an index of maximal mitochondrial electron transport capacity.

State 3, state 4 and FCCP-uncoupled respiration rates were expressed as nmol $O_2 \cdot min^{-1} \cdot$ mg mitochondrial $protein^{-1}$. They were measured at least 4 times for each mitochondrial specimen, respectively, and the averages were taken.

Mitochondrial protein assay

Mitochondrial protein concentration was measured for each specimen by the Lowry meth-

od(Markwell et all, 1978).

Creatinine determinations and calculation of GFR

Serum and urine creatinine were measured by the Jaffe-method. GFR was calculated (at 3 h using urine volume and creatinine concentration in the last 30 min interval) from the standard formula: GFR (ml/min) = urinary creatinine (mg/ml)·urine volume (ml/min)/serum creatinine (mg/ml).

Measurements of urine sodium and potassium concentration

Urine sodium and potassium concentration were measured by flame photometry.

Morphological studies

Two rats for each group were used for these studies. One cross section (1mm thick) was made sagittally from each kidney examined and immersed immediately in a vial containing 2 ml buffered 4% glutaraldehyde. After a 2 h fixation at 4 ℃, the specimens were cut to obtain tissue blocks about 1 mm^3, postfixed in 1% OsO_4, and dehydrated in graded ethanol solutions. Following treatment with propylene oxide, the samples were embedded. Semithin sections (1-2 μm thick) stained with toluidine blue were used for routine examination. For electron microscopy, ultrathin sections (50 nm thick) were used. After staining with uranyl acetate and lead citrate, they were examined and photographed with a transmission electron microscope.

In addition, the remaining tissue samples of each kidney were fixed in formalin and processed for light microscopy.

3. Statistics

Results were expressed as means ± SEM. Comparison studies of GFR and mitochondrial respiration were carried out using an one-way analysis of variance with the Student-Neuman-Keuls test. A two-way analysis of variance and the Student-Neuman-Keuls test were utilized for the analyses of sodium and potassium concentration in urine. A probability level of $P < 0.05$ was considered as indicating statistical significance.

RESULTS

Morphologic evaluation

After 3 h *in vivo* experiments, no changes between CP and SHAM-OP animals were apparent by both light and electron microscopy. The brush border, nucleoli, mitochondria, lysosomes and smooth endoplasmic reticula from S_3 segment cells of the proximal tubule in CP-treated rats appeared normal (data not shown).

Renal function studies

These data are shown in Table 1. At the end of the 3h *in vivo* experiments, GFR showed no significant difference between CP (1.50 ± 0.13 ml/min) and SHAM-OP (1.87 ± 0.08 ml/min) groups ($P > 0.05$).

Thirty min following cisplatin administration, CP-treated animals showed a 56% continuous increase of sodium concentration in urine throughout the 3h period as compared with the SHAM-OP group ($P < 0.01$). Within both groups at various intervals, no significant difference was found in urine sodium concentration ($P > 0.05$).

Urine potassium concentration of the CP group increased to 260% that of SHAM-OP group the 30 min after cisplatin injection ($P < 0.01$) and remained high during the *in vivo* experiments. There was also no significantly different urine potassium concentration within both groups at various intervals ($P > 0.05$).

Renal mitochondrial respiration studies

The results are presented in Table 2. There were no significant differences in mitochondrial respiration between control and SHAM-OP groups ($P > 0.05$). However, in CP-treated rats, 3h following cisplatin administration, state 4 respiration increased 200% that of control values ($P < 0.01$); on the contrary, state 3 respiration declined 46% ($P < 0.01$), RCR 74% ($P < 0.01$) and FCCP-uncoupled respiration 47% ($P < 0.01$), respectively, as compared to controls.

Table 1 Sodium and potassium concentrations in urine throughout the 3h *in vivo* experiments

Time (h)	Urine sodium concentration (mmol/L)		Urine potassium concentration (mmol/L)	
	SHAM-OP	CP	SHAM-OP	CP
0.5	106.38 ± 6.97	137.26 ± 16.27*	29.34 ± 3.03	126.74 ± 4.64*
1.0	96.80 ± 6.55	166.88 ± 16.07*	31.58 ± 3.74	126.42 ± 5.71*
1.5	96.36 ± 4.66	174.06 ± 16.29*	34.76 ± 4.67	120.86 ± 4.11*
2.0	101.52 ± 7.16	167.04 ± 13.12*	35.80 ± 4.14	127.74 ± 3.16*
2.5	102.76 ± 6.78	158.20 ± 2.85*	38.92 ± 4.34	128.44 ± 5.71*
3.0	106.88 ± 6.87	149.96 ± 11.47*	42.36 ± 3.34	130.42 ± 8.54*

All values were means ± SEM. SHAM-OP: sham-operated;
CP: 10mg/kg cisplatin; * $P < 0.01$ compared with sham-operated animals.

Table 2 Renal mitochondrial respiration studies at termination of the 3h *in vivo* experiments

Group	State 3 respiration (nmol·min^{-1}·mg mito protein^{-1})	State 4 respiration (nmol·min^{-1}·mg mito protein^{-1}	RCR	FCCP-uncoupled respiration (nmol·min^{-1}·mg mito protein^{-1})
Control	1069.82 ± 29.88	145.84 ± 6.04	7.45 ± 0.16	964.76 ± 36.67
SHAM-OP	967.64 ± 37.61	135.36 ± 6.03	7.21 ± 0.17	882.59 ± 22.52
CP	574.55 ± 11.63*	293.64 ± 9.39*	1.97 ± 0.05*	512.23 ± 18.02*

All values are means ± SEM. RCR: respiratory control ratio; FCCP: carbonyl cyanide p-trifluoromethoxyphenyl hydrazone; SHAM-OP: Sham-operated; CP: 10 mg/kg cisplatin; mito: mitochondrial; * $P < 0.01$ versus control animals.

DISCUSSION

Kidney proximal tubular necrosis following cisplatin administration had been well demonstrated in most animal species (Jones et al., 1985; Chopra et al., 1982). The earliest morphologic alterations were observed 6h following cisplatin administration (10 mg/kg, intraperitoneally) to rats, including nucleolar segregration, ribosome dispersion and the formation of aggregates of smooth endoplasmic reticulum throughout the S_3 segment cells of the proximal tubules (Jones et al., 1985). In sharp contrast to the variety of lesions induced by cisplatin in the renal tubules, glomerular ultrastructure appeared normal (Lauren et al., 1988). In the present study in rats, 3h after cisplatin injection (10 mg/kg, intravenously), renal morphology was indistinguishable from that of sham-operated animals by both light and electron microscopy, confirming that cisplatin appeared to have no influence on renal structure at the very early stage of this nephrotoxicity *in vivo*.

No significant changes are observed in RBF and GFR 0-4h following cisplatin administration in dogs (Daugaard, 1990). The present data were in agreement with these descriptions, indicating that cisplatin had no acute effect on GFR.

Immediately after administration of cisplatin to dogs and rats, a significant reduction in absolute proximal reabsorption rates and an increase in sodium and potassium clearance were observed (Daugaard, 1990). Some other reports, however, said that the increase in sodium and potassium excretions occured only after 72-120 h following 5-10 mg/kg cisplatin injection to rats (Jones et al., 1985). The present study results accocd with data reported by Daugaard. Thirty min following 10 mg/kg cisplatin administration to rats, urine sodium and potassium concentrations increased by 56% and 260% that of sham-operated levels, respectively, confirming the direct injurious effect by cisplatin on the renal tubules. The decrease of proximal reabsorption causes an increase of sodium and water delivery into the more distal segments of the nephron, resulting in a rise in sodium and water reabsorption

at nephron sites beyond the proximal tubules. The rise in reabsorption rates of the distal nephron segments did not completely counteract the increase in delivery from the proximal tubules. It therefore may account for the measured increase in sodium excretion rates, and the sodium load-dependent potassium secretion seems a likely explanation for the concomittant increased potassium excretion.

The precise cellular mechanism of cisplatin-induced acute renal failure is still unknown. Some studies focused on renal mitochondrial respiration abnormalities. Morphologically, mitochondrial aberrance was evident 72-96 h following a single 5.5-10 mg/kg dose of cisplatin in to rats or mice(Jones et al., 1985, Chopra et al., 1982). Brady et al. demonstrated that before a reduction in cell ATPase activity and net loss of potassium to the extracellular fluid in rabbit proximal tubule suspensions incubated with 10^{-3} mol/L cisplatin, state 4 and state 3 respiration were inhibited by 13.1% and 12.5% that of the control's respectively and that carbonyl-cyanide-m-chlorophenylhydrazone (CCCP)-uncoupled respiration declined by 26.3% that of control (Brady et al., 1990). However, Safirstein et al. found that state 4 and CCCP-uncoupled respiration in tubules isolated from rats were normal up to 96 h following a nephrotoxic dose of cisplatin, while plasma urea concentration was already elevated(Safirstein et al., 1987). The explanation of these striking discrepancies could be different methodologies, different experimental conditions, different routes of administration or different animal species, and be further complicated by the morphological and metabolic heterogeneity of the kidney and by the different susceptibilities of the various nephron segments to injury. In the present study, while no abnormalities were found in renal morphology and GFR 3 h following 10 mg/kg intravenous cisplatin injection to rats, state 4 respiration increased by 100%, but state 3 respiration, RCR and FCCP-uncoupled respiration declined by 46%, 74% and 47% that of the control's, respectively, indicating that both mitochondrial electron transport and oxidative phosphorylation are inhibited, thereby disrupting renal cell energy production. This observation strongly suggests that mitochondrial dysfunction may be a very early cellular event in cisplatin-induced renal damage *in vivo*.

The oxygen consumption per gram of tissue is very high in the kidney, being exceeded only by that of the beating heart. A sharp drop in oxygen tension occurs between the outer and inner medulla. The proximal tubule has high metabolic ATP demand and relies almost exclusively on mitochondrial oxidation phosphorylation for ATP synthesis(Silva, 1987). In the light of these and the present data, the following hypothesis could be presumed: Cisplatin-induced acute renal failure might be due to the inhibition of the mitochondrial electron transport-oxidative phosphorylation sequence in renal proximal tubules, resulting in the decline of cellular ATP levels. As cellular ATP levels diminish, anaerobic glycolysis is activated in an attempt to maintain normal cellular function. Lactic acid levels in the cytosol increase, reducing the intracellular pH. The duration and severity of cellular ATP depletion initiates a cascade of events leading to further cellular dysfunctions such as ATPase activities. The deenergized cells can no longer maintain their normal intracellular ionic environments and begin to swell, giving rise to the loss of cell polarity and cell volume regulation. Mitochondria swell and lose calcium homeostasis. The cell membrane of swollen cells become more permeable, leading to the leakage of various soluble enzymes, coenzymes and other cell constituents from the cell. As the intracellular pH drops below certain limit, damage occurs to lysosomal membranes, which release various hydrolytic proteases, lipases, glucosidases and phosphatases into the cell, bringing about autophagia of cellular components and eventually tubular necrosis. However, there are many uncertainties about this hypothesis and further investigations are needed to clarify it.

ACKNOWLEDGEMENTS

We appreciate greatly the technical assistance of Anke Jansen and Sigrid Schwabe in sev-

eral aspects of the work. We thank Dr. Leh and Mrs. Bracker for preparation and examination of light and electron microcopy.

References

Brady, H. R., Kone, B. C., Stromski, M. E. et al., 1990. Mitochondrial injury: an early event in cisplatin toxicity to renal proximal tubules. *Am J Physiol* **258**: F1181.

Chopra, S., Kaufman, J. S., Jones, T. W. et al., 1982. Cis-diamminedichlorplatinum-induced acute renal failure in the rat. *Kidney Int* **21**:54.

Daugaard, G., 1990. Cisplatin nephrotoxicity: experimental and clinical studies. *Dan Med Bull* **37**:1.

Goldstein, L., 1975. Glutamine transport by mitochondria isolated from normal and acidotic rats. *Am J Physiol* **229**:1027.

Jones, T. W., Chopra, S., Kaufman J. S. et al., 1985. Cis-diamminedichloroplatinum (II)-induced acute renal failure in the rat. Correlation of structural and functional alterations. *Lab Invest* **52**:363.

Laurent, G., Yernaux, V., Nonclercq, D. et al., 1988. Tissue injury and proliferative response induced in rat kidney by cis-diamminedichloroplatinum(II). *Virchows Arch (Cell Pathol)*:**55**:129.

Markwell, M. A. K., Haas, S. M., Bieber, L. L. et al., 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* **87**:206.

Offerman, J. J. G., Meijer, S., Sleifer, D. Th. et al., 1984. Acute effects of cis-diamminedichloroplatinum (CDDP) on renal function. *Cancer Chemother Pharmacol* **12**:36.

Safirstein, R., Winston, J., Moel, D. et al., 1987. Cisplatin nephrotoxicity: insights into mechanism. *Int J Androl* **10**:325.

Silva, P., 1987. Renal fuel utilization, energy requirements, and function. *Kidney Int* **32**:S9.