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# Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat

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**Abstract**

Cisplatin is an effective anticancer drug, but has its severe adverse effects, especially nephrotoxicity. The molecular mechanism of cisplatin-induced nephrotoxicity is still not clear. In the present study, we examined the role of rat (r)OCT2, an organic cation transporter predominantly expressed in the kidney, in the tubular toxicity of cisplatin. Using HEK293 cells stably expressing rOCT2 (HEK-rOCT2), we evaluated the cisplatin-induced release of lactate dehydrogenase and the uptake of cisplatin. The release of lactate dehydrogenase and the accumulation of platinum were greater in HEK-rOCT2 cells treated with cisplatin than in mock-transfected cells. Moreover, cimetidine and corticosterone, OCT2 inhibitors, inhibited the cytotoxicity and the transport of cisplatin in HEK-rOCT2 cells. Pharmacokinetics of cisplatin was investigated in male and female rats because the renal expression level of rOCT2 was higher in male than female rats. The renal uptake clearance of cisplatin was greater in male than female rats, while the hepatic uptake clearance was similar between the sexes. In addition, glomerular filtration rate and liver function were unchanged, but *N*-acetyl-β-D-glucosaminidase activity in the bladder urine and the urine volume were markedly increased 2 days after the administration of 2 mg/kg of cisplatin in male rats. Moreover, cisplatin did not induce the elevation of urinary *N*-acetyl-β-D-glucosaminidase activity in the castrated male rats whose renal rOCT2 level was lower than that of the sham-operated rats. In conclusion, the present results indicated that renal rOCT2 expression was the major determinant of cisplatin-induced tubular toxicity.


*Keywords:* Cisplatin; Organic cation transporter 2; Nephrotoxicity; Cytotoxicity; Renal tubular epithelial cells; Pharmacokinetics

## 1. Introduction

*cis*-Diamminedichloroplatinum II (CDDP, cisplatin) is widely used to treat solid tumors of prostate, bladder, colon, lung, testis and brain. Although cisplatin is an effective anticancer agent, severe nephrotoxicity limits its clinical application. It was reported that an increase in the serum creatinine concentration was observed in 41% of patients treated with high-dose cisplatin [1]. However, the major site of cisplatin-induced renal injury is the proximal tubule [2]. In addition, cisplatin induced tubular toxicity, followed by an increase in the serum creatinine level [3]. Moreover, the tubular toxicity caused a decrease in the glomerular filtration rate (GFR), resulting in acute renal failure [4]. Therefore, it was suggested that cisplatin was toxic primarily to renal tubular epithelial cells. But, the molecular mechanism of cisplatin-induced nephrotoxicity is still unknown.

Safirstein et al. [5] reported that cisplatin was concentrated in rat renal cortical slices five-fold above the concentration in medium. We previously demonstrated that cisplatin treatment from the basolateral side caused sever toxicity compared to the apical side in the porcine derived epithelial cell line LLC-$PK_1$ cells [6]. Recently, Ludwig et al. [7] reported that cisplatin-induced cytotoxicity was specifically observed from the basolateral side, and the toxicity was ameliorated in the presence of cimetidine in

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Madin-Darby canine kidney (MDCK) cells. These reports suggested that the uptake of cisplatin in tubular epithelial cells was mediated by basolateral drug transporter(s). Identification of the transporter(s) is essential to understand the mechanism of cisplatin-induced nephrotoxicity.

Human organic cation transporter 2 (hOCT2) is the most abundant organic cation transporter in the kidney among an organic cation transporter family which consists of hOCT1-3 (SLC22A1-3) and hOCTN1 and 2 (SLC22A4 and 5) [8,9]. Rat (r)OCT2 is expressed predominantly in the basolateral membranes of proximal tubules and mediated the accumulation of various cationic drugs into proximal tubular epithelial cells from the circulation [10–14]. Uptake of tetraethylammonium (TEA) by rOCT2 was suppressed by the replacement of $Na^+$ with $K^+$, suggesting that the transport activity of rOCT2 was membrane potential-dependent [9,15]. Based on such backgrounds and findings, we hypothesized that rOCT2 was the key molecule to clarifying the tubular accumulation and subsequent nephrotoxicity of cisplatin.

In the present study, we investigated whether rOCT2 affected the nephrotoxicity of cisplatin in rat proximal tubules. We examined the effect of rOCT2 expression on the cytotoxicity of cisplatin in HEK293 transfectants and on the pharmacokinetics of cisplatin in rats.

## 2. Materials and methods

### 2.1. Cell culture and transfection

HEK293 cells (American Type Culture Collection CRL-1573) were cultured in complete medium consisting of Dulbecco's modified Eagle's medium (Sigma Chemical Co., St. Louis, MO) with 10% fetal bovine serum (Whittaker Bioproducts Inc., St. Louis, MO) in an atmosphere of 5% $CO_2$–95% air at 37 °C.

The construction of HEK293 cells stably expressing rOCT2 (HEK-rOCT2) was performed as described [14]. The transfectants were used for the experiments at 48 h after seeding.

For a transient expression system, pBK-CMV plasmid vector DNA (Stratagene, La Jolla, CA), containing rOCT1 or rOCT2 cDNA, was purified using Wizard® Plus SV Minipreps DNA Purification System (Promega, San Luis Obispo, CA). The day before transfection, HEK293 cells were seeded onto poly-D-lysine-coated 24-well plates at a density of $2.0 \times 10^5$ cells per well. The cells were transfected with 50 ng of total plasmid DNA per well using 0.125 μl of LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) per well according to the manufacturer's instructions. Forty-eight hours after the transfection, the cells were used for uptake experiments.

### 2.2. Uptake experiment

Cellular uptake of [$^{14}$C]TEA (88.8 MBq/mmol, Perkin-Elmer Inc., Wellesley, MA) was measured with monolayer cultures grown on poly-D-lysine-coated 24-well plates. The composition of the incubation buffer was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose and 5 mM HEPES (pH 7.4 adjusted with NaOH). Experimental procedures were performed as described previously [14].

For the measurement of cisplatin uptake, seeded cells were incubated with the medium containing cisplatin with or without cimetidine or corticosterone for 1 h. After this incubation, the monolayers were rapidly washed twice with ice-cold incubation buffer containing 1% bovine serum albumin (Nacalai Tesque, Kyoto, Japan) and then washed three times with ice-cold incubation buffer. The cells were solubilized in 0.5N NaOH, and the amount of platinum was determined using inductively coupled plasma-mass spectrometry (ICP-MS) by the Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd. (Wakayama, Japan).

The protein content of the cell monolayers solubilized in 0.5N NaOH was determined by the method of Bradford with a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA).

### 2.3. Cytotoxicity experiment

The cytotoxicity of cisplatin was measured with monolayer cultures grown on poly-D-lysine-coated 12-well plates. Cells were incubated with the medium containing cisplatin with or without cimetidine or corticosterone for 2 h. After removal of the medium, drug-free medium was added. After incubation for 24 h, the medium was collected, and the lactate dehydrogenase (LDH) activity in the medium was measured using a LDH Cytotoxicity Detection Kit (Takara, Shiga, Japan), according to the manufacturer's instructions. Cytotoxicity was evaluated by measuring LDH activity in the medium. Total LDH activity was defined as LDH activity in the medium containing 1% TritonX-100. LDH release represents (LDH activity − LDH activity of control)/(total LDH activity − LDH activity of control).

### 2.4. Quantification of mRNA expression

Cellular total RNA was extracted using a MagNA Pure LC RNA isolation kit II (Roche Diagnostic GmbH, Mannheim, Germany) [8]. The total RNA was reverse-transcribed, and the single stranded DNA was used for the quantification of mRNA expression.

Real-time PCR was performed in a total volume of 20 μl containing 2 μl of reverse-transcribed cDNA, 1 μM forward and reverse primers, 0.2 μM TaqMan probe, and 10 μl of TaqMan Universal PCR Master Mix (Applied