

Enhanced renal accumulation of cisplatin via renal organic cation transporter deteriorates acute kidney injury in hypomagnesemic rats

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Abstract

Background This study aimed to explore the effects of hypomagnesemia on cisplatin (CDDP)-induced acute kidney injury (AKI) in rats and the relation of hypomagnesemia to the regulation of organic cation transporters and renal accumulation of CDDP.

Methods Sprague–Dawley rats were given an Mg-deficient diet starting 7 days before treatment with CDDP. CDDP was administered intravenously to rats in the normal Mg-diet group and Mg-deficient-diet group at 3 mg/kg via the left jugular vein. At the specified periods after injection of CDDP, the amount of platinum in blood and organ samples was determined using inductively coupled plasma-mass spectrometry. Protein expression levels of renal organic cation transporters were determined. Uptake of tetraethylammonium (TEA) bromide in renal slices of rats was measured.

Results Rats fed a Mg-deficient diet showed a significant body weight decrease and a marked decrease in serum Mg levels compared with control rats fed an adequate Mg diet. Serum blood urea nitrogen and creatinine levels were unaltered after CDDP treatment in control rats, whereas these levels were markedly elevated in hypomagnesemic rats. Immunoblotting revealed up-regulation of the organic

cation transporter rOCT2 in hypomagnesemic rats before CDDP administration, but not of rOCT1 or rat multidrug and toxin-extrusion 1. TEA uptake by renal slices from hypomagnesemic rats was significantly higher compared with that of control rats. Renal accumulation of CDDP was markedly increased in hypomagnesemic rats.

Conclusion These results suggest that hypomagnesemia could cause dehydration and up-regulation of rOCT2, enhancing renal accumulation of CDDP and the deterioration of AKI.

Keywords Cisplatin · Hypomagnesemia · Acute kidney injury · Organic cation transporter

Introduction

Cisplatin (*cis*-diammine-dichloroplatinum; CDDP), an inorganic platinum chemotherapeutic drug, has been widely used in the clinical treatment of various solid tumors [1, 2]. Although CDDP has potent antitumor efficacy and leads to a significant improvement in survival rates of cancer patients, several adverse reactions, including acute and chronic kidney injuries, myelosuppression, ototoxicity, and neurotoxicity, are dose-limiting factors [3]. Among these adverse effects, acute kidney injury (AKI) is recognized to be the most frequent and severe toxicity.

CDDP is predominantly eliminated in urine via both glomerular filtration and renal secretion in the proximal tubules. CDDP is suggested to enter renal tubular cells through passive diffusion with active uptake mediated by basolaterally localized organic cation transporter(s). Rat organic cation transporters, rOCT1 and rOCT2, are driven electrogenetically by inside-negative membrane potentials, thereby mediating basolateral accumulation of diverse

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organic cations into renal cells [4]. In contrast, rat multi-drug and toxin extrusion (rMATE) 1, which mediates the exchange of organic cations with hydrogen ions in renal brush-border membranes, is suggested to be responsible for the final step of urinary excretion of cationic drugs [5, 6]. In CDDP-induced AKI, a recent report suggested that rOCT2 was the predominant transporter involved in the renal cumulative uptake of CDDP in rats [7].

CDDP-induced AKI is accompanied by disturbances in renal handling of electrolytes. In particular, hypomagnesemia has emerged as a common event associated with CDDP-induced AKI; 76% of patients became hypomagnesemic during CDDP treatment [8, 9]. However, there is a paucity of information concerning the pathogenesis and mechanisms underlying CDDP-associated hypomagnesemia and associated kidney injuries. Mavichak et al. [10] reported that abnormalities of magnesium (Mg) metabolism were induced by CDDP administration in rats. In addition, abnormal renal handling of Mg persisted along with residual lesions in the S3 segment of the outer medullary nephrons, indicating that renal tubular reabsorption of filtered Mg is suppressed under CDDP-induced AKI [11]. Alternatively, hypomagnesemia appeared to be involved in further deterioration of renal tubular dysfunction following treatment with CDDP. Lajer et al. [12] reported that significant derangements of renal function were associated with increases in serum creatinine (SCr) and blood urea nitrogen (BUN) levels in CDDP-treated rats fed a Mg-deficient diet, that is, almost complete Mg depletion compared with an adequate Mg diet. These results suggest that the decrease in serum Mg levels is responsible for the deterioration and/or progression of AKI caused by CDDP. However, there is little information concerning the mechanisms of deterioration and/or progression of CDDP-induced AKI under conditions of hypomagnesemia. Here, we report the possible mechanisms involved in the development of CDDP-evoked AKI under conditions of hypomagnesemia in relation to renal epithelial uptake and accumulation of CDDP via organic cation transporters.

Materials and methods

Chemicals

CDDP was a kind gift from the Nihon Kayaku Co. (Tokyo, Japan). D-[1-³H(N)]Mannitol (525.4 GBq/mmol) and [1-¹⁴C]TEA (118.4 MBq/mmol) were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). The adequate Mg diet contained (g/kg) milk casein 245, granulated sugar 100, cornstarch 455, fiber (cellulose) 30, AIN-93 mineral mix 70 (3.5 Mg), and AIN-93 vitamin mix 10. The Mg-deficient diet contained the same ingredients above without Mg (Clea Japan, Inc., Tokyo, Japan).

Cimetidine was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were commercially available products and of reagent grade.

Animals

Male Sprague–Dawley rats (5 weeks old) (Clea Japan, Inc.) were housed in a standard animal maintenance facility at constant temperature (21–23°C) for at least 1 week before the day of the experiment. All animal experiments were conducted according to the guidelines of Kumamoto University for the Care and Use of Laboratory Animals. BUN, SCr, Mg, Na, Cl, K, and Ca in serum were measured at the SRL laboratory (Tokyo, Japan).

Pharmacokinetic analysis of CDDP

Rats in the Mg-deficient diet group were given an Mg-deficient diet starting 7 days before treatment with CDDP. CDDP (3 mg/kg) was administered intravenously to rats in the normal Mg-diet group and Mg-deficient-diet group via the left jugular vein over about 1 min. Blood samples were collected from the right jugular vein at 5 and 30 min and 1, 2, 4, 8, 12, 24, 72, 120, and 168 h after injection of CDDP. Blood samples were centrifuged at 3,000 g for 5 min. Plasma samples were stored at –80°C until analysis. At 24, 72, 120, and 168 h after injection of CDDP, blood, liver, and kidney samples were collected immediately after the rats were anesthetized with ether and killed. Serum samples were stored at –80°C until analysis. Organ samples were homogenized in 0.5% nitric acid (HNO₃). Each sample was incinerated using a super-high frequency sample degradation container (DV-7; Sanai Kagaku, Nagoya, Japan) encased with Teflon and propylene outer casing (PP-25 and PT-25, Sanai Kagaku) in a microwave oven with 0.5% HNO₃. Plasma and the incinerated samples were diluted to HNO₃. The amount of platinum was determined using inductively coupled plasma-mass spectrometry (ICP-MS; Finnigan MAT ELEMENT, Bremen, Germany). The K_p value for tissue accumulation of ¹⁹⁴Pt was calculated by dividing the tissue concentration of ¹⁹⁴Pt by the serum concentration of ¹⁹⁴Pt. The pharmacokinetic parameters, such as the area under the serum concentration-time curve, distribution volume, and total clearance for serum CDDP concentration profiles, were estimated with the WinNonlin version 3.1 software using a non-compartment model.

Western blot analysis

Kidneys were homogenized in a homogenization buffer comprising 230 mM sucrose, 5 mM Tris–HCl, pH 7.5, 2 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride,

1 mg/ml leupeptin, and 1 mg/ml pepstatin A. After measurement of the protein content using bicinchoninic acid (BCA) protein assay reagent (Pierce, Rockford, IL), each sample was mixed in a loading buffer [2% sodium dodecyl sulfate-polyacrylamide (SDS), 125 mM Tris-HCl, 20% glycerol, 5% 2-mercaptoethanol] and heated at 100°C for 2 min. The samples were separated by 7.5% SDS gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore, Bedford, MA) by semi-dry electroblotting. The blots were blocked overnight at 4°C with 2% ECL advance blocking agents (GE Healthcare Bio-sciences Corp., Piscataway, NJ) in Tris-buffered saline (TBS) containing 0.3% Tween 20 (TBS-T) and incubated for 1 h at room temperature with a primary antibody specific for rOCT1, rOCT2, and rMATE. The blots were washed with TBS-T and incubated with the secondary antibody horseradish peroxidase linked anti-rabbit immunoglobulin F (ab)₂ (GE Healthcare Bio-Sciences, Piscataway, NJ) for 1 h at room temperature. Immunoblots were visualized with an ECL system (ECL Advance Western Blotting Detection Kit, GE Healthcare Bio-Sciences, Piscataway, NJ).

Uptake assay by renal slices

Uptake studies using isolated rat renal slices were carried out as described in a previous report [13]. Briefly, slices of kidney were stored in ice-cold oxygenated incubation buffer composed of 120 mM NaCl, 16.2 mM KCl, 1 mM CaCl₂, 1.2 mM MgSO₄, and 10 mM NaH₂PO₄/Na₂HPO₄, pH 7.5. Renal slices were randomly selected and placed for incubation in flasks containing 6 ml of the incubation buffer with [¹⁴C]TEA (5 μM, 0.56 kBq/ml). The uptake of these compounds was carried out at 37°C under an atmosphere of 100% oxygen for 60 min. D-[³H]Mannitol (5 μM, 1.85 kBq/ml) was used to calculate the extracellular trapping and non-specific uptake of [¹⁴C]TEA as well as to evaluate the viability of slices. After incubation, the incubation buffer containing radiolabeled compounds was rapidly removed from the flask, washed twice with 5 ml of ice-cold phosphate buffered saline, blotted onto filter paper, weighed, and solubilized in 0.5 ml of NCSII (GE Healthcare Bio-Sciences, Piscataway, NJ). The amount of radioactivity was then determined in a liquid scintillation counter after adding 5 ml of OCS (GE Healthcare Bio-Sciences, Piscataway, NJ).

Statistical analysis

Differences between groups were analyzed using the unpaired Student's *t* test. A value of *P* < 0.05 was considered significant.

Results

To investigate the effect of hypomagnesemia on the CDDP-induced AKI, we first examined whether hypomagnesemia with CDDP could induce AKI in rats. Rats were divided into two groups as follows: the control group and the Mg-deficient diet group (low Mg group) were given the normal Mg diet and the Mg-deficient diet starting 7 days before treatment with CDDP (3 mg/kg). Figure 1 shows changes in the absolute body weight and renal function of the rat control group and low Mg group. The body weight of low Mg group decreased significantly at 0 h before administration of CDDP compared to that of the control group (Fig. 1a). Thereafter, the body weight of control rats significantly increased, whereas that of the low Mg group was unchanged, suggesting that Mg depletion caused a decrease in intake of diet and/or water. Levels of BUN and SCr were slightly elevated at 72 h in control rats (Fig. 1b, c). In the low Mg group, CDDP significantly increased levels of BUN and SCr at 120 h, and the elevated BUN and SCr were partially depressed at 168 h. Serum electrolytes at 0 and 120 h are shown in Table 1. Serum Mg levels of low Mg rats were significantly much lower than that of control rats at 0 and 120 h. Except for Mg, serum electrolytes were unchanged by the Mg-deficient diet and/or administration of CDDP.

We measured the distribution of CDDP after intravenous administration of CDDP (3 mg/kg) using ICP-MS. Figure 2 shows the plasma concentration-time profiles of ¹⁹⁴Pt after the administration of CDDP in control and low Mg diet rats. Plasma concentrations of ¹⁹⁴Pt in low Mg diet rats were not significantly different from concentrations in control rats. Pharmacokinetic parameters for plasma ¹⁹⁴Pt profiles were not different between the two groups (data not shown). In contrast, renal accumulation of CDDP in low Mg diet rats was significantly elevated about two-fold at 24 h after the administration of CDDP compared with control rats (Table 2). The differences in renal accumulation of CDDP were sustained for 24–168 h. In contrast, the accumulation of CDDP in the liver was not affected by the Mg-deficient diet at 24 h after the administration of CDDP. ¹⁹⁴Pt(CDDP) accumulations in the liver at 72, 120 and 168 h after the CDDP treatment were significantly elevated (Table 2). The mechanism for the increased levels of hepatic ¹⁹⁴Pt remains unknown. It could be speculated that AKI caused by CDDP would affect hepatic uptake and/or bile excretion of the drug, or uremic toxins associated with renal injury may be involved in the altered hepatic accumulation and/or disposition of CDDP.

We next examined the impact of the Mg-deficient diet and CDDP treatment on renal expression levels of organic cation transporters rOCT1, rOCT2, and rMATE1 in the

Fig. 1 Changes in body weight and renal function data in the control group and low Mg group. Body weight (a), BUN (b), and SCr (c) after intravenous administration of cisplatin (3 mg/kg) in the control group (open column) and low Mg group (closed column) are shown. Each point represents the mean ± SD from three rats. **P* < 0.05, ***P* < 0.01, significantly different from the control group at the same period. †*P* < 0.05, ††*P* < 0.01, significantly different from the control group at 0 h. #*P* < 0.05, ##*P* < 0.01, significantly different from the low Mg group at 0 h

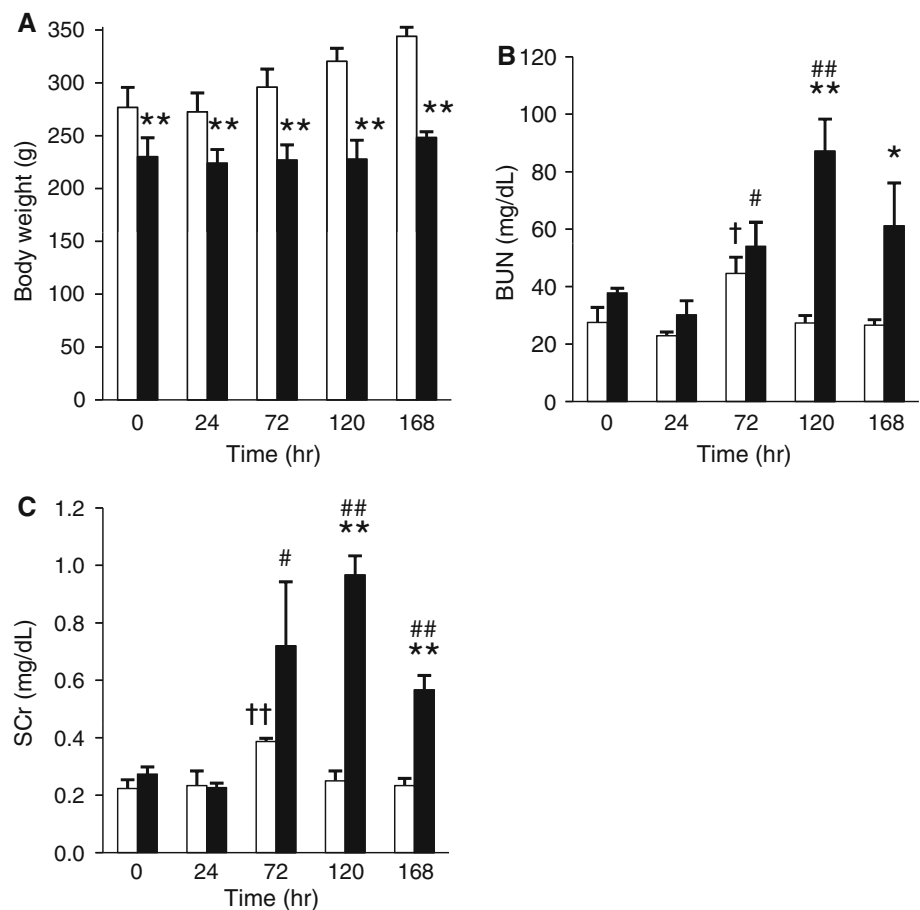


Table 1 Level of serum electrolytes in rats fed an adequate Mg diet or Mg-deficient diet

	0 h		120 h	
	Adequate Mg	Mg-deficient	Adequate Mg	Mg-deficient
Mg (mEq/dl)	2.2 ± 0.1	0.5 ± 0.1**	2.3 ± 0.2	0.7 ± 0.0**##
Na (mEq/dl)	132.3 ± 9.1	141.0 ± 1.0	141.7 ± 0.6	141.3 ± 4.0
Cl (mEq/dl)	93.3 ± 7.5	105.0 ± 0.0	100.7 ± 0.6	100.0 ± 3.5
K (mEq/dl)	4.8 ± 0.6	5.2 ± 0.2	5.1 ± 0.8	4.4 ± 0.3
Ca (mEq/dl)	9.9 ± 0.7	10.5 ± 0.3	9.9 ± 0.3	9.6 ± 1.4

Each value represents the mean ± SD from three rats

** *P* < 0.01, significantly different from the control group at the same period

P < 0.01, significantly different from the low Mg group at 0 h

control group and low Mg group by Western blot analysis (Fig. 3a). Densitometric analysis revealed that the rOCT1 expression level was unchanged by the Mg-deficient diet and/or administration of CDDP (Fig. 3b). In contrast, rOCT2 expression levels in the low Mg group at 0 h were significantly increased compared with control rats at 0 h (Fig. 3c). Furthermore, rOCT2 expression levels in the control group at 120 h were not affected by the administration of CDDP, whereas levels in the low Mg group at

120 h were considerably reduced. rMATE1 expression was significantly up-regulated at 120 h after CDDP treatment in the low Mg group (Fig. 3d).

To evaluate the functional activity of organic cation transporter(s) at the basolateral membranes, we measured the accumulation of TEA, a typical substrate for organic cation transporters, in renal slices prepared from the control and low Mg group rat kidneys. As shown in Fig. 4, the accumulation of TEA in renal slices from the low Mg

group was about two-fold significantly higher than that in renal slices from control rats.

Discussion

CDDP-induced AKI is a well-known adverse effect of chemotherapy. This severe nephrotoxicity is associated with a disturbance in the homeostasis of Mg. Alternatively, hypomagnesemia enhances CDDP-induced nephrotoxicity. However, there is a paucity of information concerning the precise mechanisms for enhancing CDDP-induced nephrotoxicity under hypomagnesemia. In this study, we

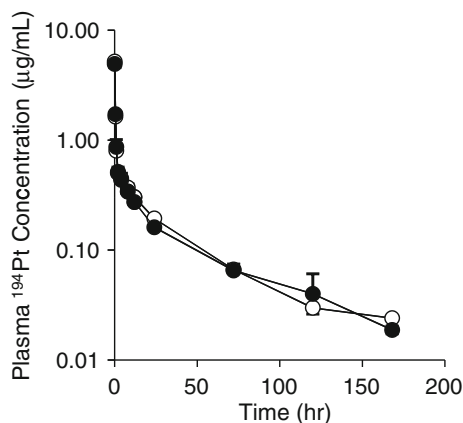


Fig. 2 Plasma concentrations of ^{194}Pt versus time in the control group (open circle) and low Mg group (closed circle) after intravenous administration of cisplatin (3 mg/kg). Each point represents the mean \pm SD from three rats

investigated the development of CDDP-induced AKI under hypomagnesemia, that is, we used a Mg-depleted rat model to examine the renal accumulation process of CDDP via renal plasma membrane-specific transporters.

A marked depression in serum Mg levels was confirmed in the hypomagnesemic rats fed the Mg-deficient diet. Levels of BUN and SCr were significantly elevated by a single intravenous administration of CDDP (3 mg/kg) in low Mg diet rats, but not in control rats, at 120 h after the treatment with CDDP. Namely, AKI was induced by CDDP in low Mg diet rats. In addition, Mg depletion suppressed an increase in the body weight, suggesting a decrease in intake of diet and/or water, i.e., dehydration. Therefore, Mg-evoked dehydration could be a risk factor for the development and further deterioration of CDDP-induced AKI in this experimental condition. In a previous study, enhancement of CDDP-induced AKI was caused by inflammatory cytokines and oxidative stress that appeared to be evoked in response to Mg depletion [14–16]. Furthermore, it was reported that Mg supplementation prevented cyclosporine-induced chronic kidney injury [17]. Considering these findings, Mg depression in serum could be one of the risk factors involved in the development of CDDP-induced AKI. It was suspected that low Mg conditions would have an effect on renal handling of CDDP, as rats in the low Mg group showed a two-fold increase in renal accumulation of CDDP. In fact, we found that the uptake of TEA by renal slices was increased about two-fold in rats fed the Mg-deficient diet. Therefore, it is suggested that Mg depression stimulates renal uptake of CDDP via the basolateral organic cation transport system. A previous study demonstrated that rOCT2 played a major role in the

Table 2 The tissue distribution of ^{194}Pt in control and low Mg group after i.v. administration of cisplatin (3 mg/kg)

	Control		Low Mg	
	Concentration ($\mu\text{g/g}$ of tissue)	Kp value (ml/g of tissue)	Concentration ($\mu\text{g/g}$ of tissue)	Kp value (ml/g of tissue)
24 h				
Kidney	3.812 ± 0.837	19.9 ± 5.9	$6.046 \pm 1.108^*$	$37.9 \pm 2.9^{**}$
Liver	0.913 ± 0.241	4.8 ± 1.7	0.962 ± 0.349	6.0 ± 1.5
72 h				
Kidney	3.303 ± 0.279	49.8 ± 5.6	$6.980 \pm 1.080^{**}$	$110.5 \pm 35.2^*$
Liver	0.598 ± 0.183	8.9 ± 2.4	$1.014 \pm 0.137^*$	$15.7 \pm 2.4^*$
120 h				
Kidney	2.246 ± 0.154	75.7 ± 4.4	$4.200 \pm 0.979^*$	115.2 ± 30.1
Liver	0.355 ± 0.147	11.8 ± 4.5	0.780 ± 0.388	19.8 ± 2.5
168 h				
Kidney	1.517 ± 0.059	67.1 ± 21.3	$3.275 \pm 0.148^{**}$	$174.6 \pm 7.7^{**}$
Liver	0.261 ± 0.038	11.2 ± 1.8	$0.606 \pm 0.131^*$	$32.2 \pm 6.6^{**}$

Each point represents the mean \pm SD from three rats

* $P < 0.05$; ** $P < 0.01$, significantly different from the control group at the same period

Fig. 3 Protein expression levels of transporters in the kidney of the control group and low Mg group at 0 and 120 h after the administration of cisplatin (3 mg/kg). Immunoblotting was performed by using antiserum specific for rOCT1, rOCT2, and rMATE1 as primary antibodies (a). Relative band intensities were quantified by densitometry for the protein expression levels of rOCT1 (b), rOCT2 (c), and rMATE1 (d). Each column represents the mean \pm SD for three rats. * $P < 0.05$, ** $P < 0.01$, significantly different from the control group at the same period. † $P < 0.05$, significantly different from the control group at 0 h. ## $P < 0.01$, significantly different from the control group at 0 h

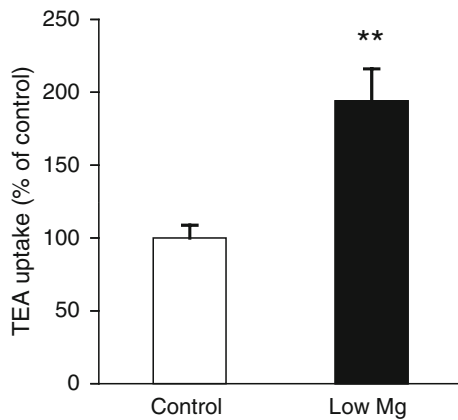
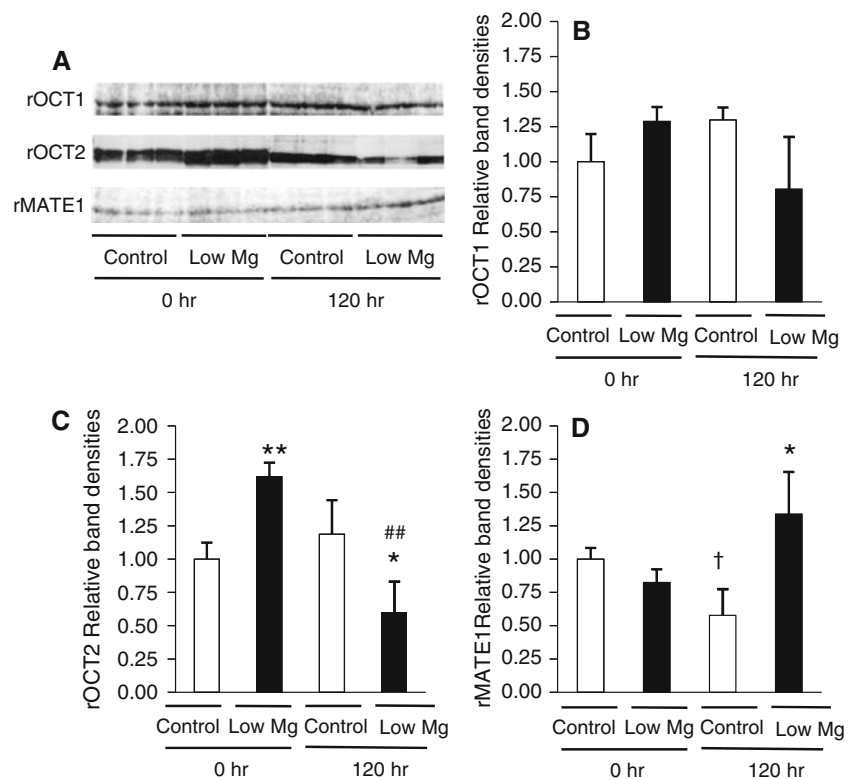


Fig. 4 Uptake of TEA in renal slices of the control group and low Mg group before CDDP administration. Data represent the mean \pm SD for 12 slices from four rats. ** $P < 0.01$, significantly different from the control group

renal distribution of CDDP, but not rOCT1 in rats [18]. Western blot analysis showed that protein expression levels of rOCT1 and rMATE1 were unchanged by the Mg-deficient diet, whereas rOCT2 expression was markedly up-regulated by the Mg-deficient diet. A previous study suggested that testosterone induced the expression of rOCT2, but not rOCT1, via the androgen receptor-mediated transcriptional pathway [19]. Furthermore, it is suggested that Mg affected the interconversion of androgen receptor forms [20]. Accordingly, up-regulation of rOCT2

expression might be mediated by Mg via androgen receptors. On the other hand, Mg appears to be involved in more than 300 enzymatic reactions [21]. Mg might play a role in regulating the expression of rOCT2 via other unknown mechanisms. Further studies are required to determine the involvement of Mg in up-regulation of rOCT2. On the other hand, rOCT2 expression was down-regulated 120 h after CDDP treatment in the low Mg group. We previously reported that protein expression levels and the activity of rOCT2 were markedly decreased by CDDP-induced AKI and ischemia/reperfusion-induced AKI [22, 23]. Using 5/6 nephrectomized rats, Ji et al. [24] reported that renal clearance of unbound cimetidine, a substrate of rOCT2, and the expression levels of rOCT2 were correlated. Therefore, rOCT2 appeared to be an inducible basolateral organic cation transporter that is down-regulated in association with both acute and chronic kidney injury. The serum testosterone was suggested to be one of the factors involved in rOCT2 regulation [24] and was reported to be decreased by CDDP-induced AKI [25]. Thus, testosterone could be a secondary factor for the CDDP-induced down-regulation of rOCT2.

Despite the finding that protein expression levels of rOCT2 were decreased at 120 h after administration of CDDP in low Mg diet rats, the renal Kp value was increased over time in each group. Accordingly, in the case of decreased rOCT2 activity, some transport systems different from rOCT2 might mediate renal accumulation of

CDDP. Yokoo et al. [26] suggested that CDDP was not transported by hMATE1 and hMATE2-K. CTR1 is a high-affinity copper transporter that has recently been implicated in CDDP transport [27]. In proximal tubules, CTR1 is expressed in the basolateral membrane, indicating that CTR1 contributes to accumulation of CDDP and nephrotoxicity [28]. Renal accumulation of CDDP might be predominantly mediated by OCT2 in normal kidney conditions, whereas that of CDDP could be mediated by CTR1 in the case of decreased rOCT2 activity under conditions of AKI.

In conclusion, this study reports the first evidence that Mg depletion is involved in the up-regulation of rOCT2, thereby evoking deterioration and/or progression of CDDP-induced AKI. In addition, Mg depletion could induce dehydration, which may enhance the CDDP toxicity. Our findings provide novel information for understanding the mechanisms involved in CDDP-induced AKI under hypomagnesemia and the renal accumulation of CDDP via basolateral membrane organic cation transporters in rats.

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