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Magnesium co-administration decreases cisplatin-induced nephrotoxicity in the multiple cisplatin administration☆

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ABSTRACT

Purpose: Pretreatment with magnesium (Mg) has been reported to attenuate cisplatin (CDDP)-induced nephrotoxicity (CIN). This attenuation involves modulation of the expression of renal transporters, resulting in reduced renal platinum accumulation after a single round of CDDP treatment. In this study, we investigated whether Mg co-administration ameliorates CIN after multiple doses of CDDP as effectively as after a single dose.
Methods: Rats were divided into control, Mg alone, CDDP alone, and CDDP with Mg groups. Rats received CDDP (2.5 mg/kg), $MgSO_4$ (40 mg/kg), or saline once per week for three weeks. Seven days after the third round of treatment, the kidneys were excised, and the expression of renal transporters and renal platinum accumulation were analyzed.
Results: CDDP significantly elevated serum creatinine levels, which were significantly reduced by Mg co-administration. Renal platinum accumulation was significantly lower in the CDDP-Mg group than in the CDDP group. Expression of renal organic cation transporter 2 (rOct2) and multidrug and toxin extrusion protein 1 (rMate1), which are involved in CDDP transport, did not differ between the groups. However, the expression of copper transporter 1 (rCtr1) was significantly downregulated after Mg co-administration.
Conclusion: Mg co-administration significantly attenuated CIN by reducing renal platinum accumulation even after multiple rounds of treatment with CDDP as effectively as in a model of a single CDDP administration. However, the specific underlying mechanism was different between single and multiple administrations, further studies will be needed to identify what contributes to this difference and to elucidate how Mg regulates the expression of renal transporters.

1. Introduction

Cisplatin (CDDP) is the primary drug used to treat many types of cancer. CDDP-induced nephrotoxicity (CIN) is one of the most significant adverse effects associated with CDDP treatment [1]. CIN is dose-dependent, cumulative, and is usually reversible; it occurs in 30–40% of patients who receive CDDP [2–4]. Hydration is used to prevent CIN; however, the nephroprotective effects of hydration are insufficient. Various clinical trials have reported that pretreatment with magnesium (Mg) attenuates CIN [4–7]. We and others have reported that Mg co-administration attenuates CIN by reducing platinum (Pt) accumulation in the kidney [8,9]. CDDP primarily accumulates in the S3 segment of the proximal tubule, where it is taken up by organic cation transporter 2 (rOct2). It is then transported from the proximal tubule to urine via multidrug and toxin extrusion protein 1 (rMate1) [10]. Copper transporter 1 (rCtr1) has also been reported to transport CDDP to the proximal tubule, although it is known to be a channel-like transporter [10].

Hypomagnesemia is a well-known adverse effect caused by CDDP. The expression of rOct2, which is predominantly expressed in the basolateral membranes of proximal tubules, is upregulated in rats with hypomagnesemia [11]. CDDP also regulates the expression of renal transporters, including rOct2 and rMate1 [8,9,12–14]. We have shown that CDDP administration upregulates rOct2 expression, and Mg co-

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administration regulates the expression of rOct2 and rMate1, resulting in reduced renal Pt accumulation [9]. However, all the aforementioned studies were performed using a single-dose model of CDDP, although CDDP is typically administered repeatedly in clinical settings. As previously mentioned, CIN is cumulative over the course of treatment; moreover, a single administration of CDDP not only regulates the expression of renal transporters, which have important roles in its renal accumulation, but also reduces serum levels of Mg. Therefore, it is possible that either the direct or indirect regulation of renal transporter expression by CDDP is associated with the accumulation of CIN.

In this study, we aimed to evaluate the nephroprotective effects of Mg against CIN caused by multiple rounds of CDDP administration, focusing on its effect on the expression of renal transporters and Pt accumulation.

2. Methods

2.1. Chemicals

CDDP was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). The primary anti-rOct2 and anti-rMate1 antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX, USA), anti-rCtr1 was from Abcam (Cambridge, UK), anti-rOct1 was from ABGENT (San Diego, CA, USA), and anti-rActin was from Merck Millipore (Billerica, MA, USA). Creatinine was measured using a kit (L-type Wako CRE·M, enzymatic method) purchased from Wako (Osaka, Japan). Serum Mg levels were determined using the Metallo Assay Mg LS kit from Metallogenics (Chiba, Japan). All other chemicals and reagents were commercially sourced and of the highest available purity.

2.2. Animals

Male Wistar rats (7 weeks old) weighing 210–230 g were obtained from JLA (Tokyo, Japan). All animals were housed at an animal maintenance facility in a room with controlled temperature (23 °C) and moisture (60 ± 10%) conditions and a 12 h light–dark cycle. All rats were allowed free access to demineralized water and diet pellets. All animal experiments were conducted according to the guidelines for the Care and Use of Laboratory Animals of Hokkaido University, and all experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the "Guide for the Care and Use of Laboratory Animals."

2.3. Experimental design

Rats were treated with magnesium sulfate (40 mg/kg, 16 mg/mL solution) or saline, which was intraperitoneally administered as a prophylactic agent 4 h prior to treatment with CDDP (2.5 mg/kg, 1 mg/mL solution) or saline as a therapeutic agent [9], once a week for three weeks. Rats were divided into four groups and treated as follows: (1) control group, saline prophylactically and therapeutically; (2) Mg group, Mg prophylactically and saline therapeutically; (3) CDDP group, saline prophylactically and CDDP therapeutically; and (4) CDDP-Mg group, Mg prophylactically and CDDP therapeutically.

Blood samples (300–500 μL at each time point) were collected from the tail vein at baseline (before prophylactic agent administration) and 5 and 7 days after each injection of the therapeutic agent. Body weight of rats was measured at the same time as blood sampling. After blood collection 7 days after the third injection, the rat kidneys were excised immediately. Kidney tissue and serum samples were stored at −80 °C until further analysis.

2.4. Western blot analysis

Expression of rOct2 and rMate1 was assessed by western blotting, as described previously [9]. For rCtr1 expression, samples were separated by using 15% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to membranes, which were incubated overnight with a primary antibody against rCtr1 (1:1000). For rOct1 expression, samples were incubated with a primary antibody against rOct1 (1:400). Then, the samples were visualized after the addition of a goat anti-rabbit IgG-horseradish peroxidase (HRP) secondary antibody (Santa Cruz Biotechnology; both antibodies at 1:2000).

Table 1
Primer sequences for rat kidney injury molecule-1 (rKim-1).

Genes	Primer sequence	Expected product size
rKim-1	Forward: 5′-ACCTCTACTCCAACACCAGAAC-3′ Reverse: 5′-GGCTTCCTCAAAGGGATTCTTAC-3′	185 bp

2.5. Measurement of mRNA expression

Total RNA was isolated from total protein extracts using an ISOGEN II kit (Nippon Gene, Tokyo, Japan). Total RNA was reverse transcribed using ReverTra Ace (Toyobo, Osaka, Japan) with random primers according to the manufacturer's instructions. Expression of kidney injury molecule-1 (rKim-1) mRNA was determined by RT-PCR, using an rKim-1-specific primer and a KAPATaq Extra kit (NIPPON Genetics, Tokyo, Japan); rActin was used as an internal standard. Table 1 shows the primer sequences used for the PCR amplification. The PCR thermocycling protocol was as follows: 25 cycles of denaturing at 95 °C for 30 s, annealing at 58 °C for 30 s, followed by a final extension at 72 °C for 30 s. Products were size-fractionated on agarose gel and stained using ethidium bromide.

2.6. Pt accumulation in kidney tissues

Levels of Pt in the kidney tissues were measured using inductively coupled plasma-mass spectrometry (ICP-MS) at the Creative Research Institution of Hokkaido University, as previously described [9].

2.7. Statistical analysis

Statistical analyses of data were performed using an unpaired Student's *t*-test or ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant if $P < 0.05$.

3. Results

3.1. Effects of treatments on body weight

CDDP-treated rats gained significantly less body weight than did rats in the control or Mg groups over the course of the experiment. Furthermore, Mg administration had no effect on body weight increase, regardless of CDDP administration (Table 2).

3.2. Variation in serum creatinine, Mg, and renal rKim-1 mRNA expression

Administration of CDDP significantly elevated levels of serum creatinine at all time points tested after the first injection (Fig. 1A). Interestingly, Mg co-administration with CDDP significantly attenuated changes in the levels of serum creatinine on days 8, 15, and 22. Mg administration alone had no effect on the levels of serum creatinine. Moreover, the renal mRNA expression of rKim-1, which is a primary marker of proximal tubule damage, was downregulated by Mg and CDDP co-administration compared with those observed after CDDP administration alone (Fig. 1B).

CDDP treatment significantly reduced serum Mg levels compared with the value for the control group (Fig. 2), whereas Mg co-administration with CDDP significantly improved CDDP-induced serum