European Journal of Pharmacology 811 (2017) 191–198

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Magnesium attenuates cisplatin-induced nephrotoxicity by regulating the expression of renal transporters

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ARTICLE INFO

Keywords:
Cisplatin
Nephrotoxicity
Magnesium
rOct2
rMate1
Drug transport across membranes

ABSTRACT

Cisplatin (CDDP)-induced nephrotoxicity (CIN) is one of the most serious toxicities caused by this potent antitumor agent. It has been reported that Mg premedication attenuates CIN in clinical trials; however, the mechanism underlying its nephroprotection is not fully understood. Therefore, the aim of this study was to determine whether Mg administration affects CDDP accumulation by regulating the expression level of renal transporters. Rats were divided into control, Mg (40 mg/kg) alone, 2.5 mg/kg CDDP with (20 and 40 mg/kg) and without Mg, 5 mg/kg CDDP groups. These substances were administered on the same day and 7 days later their kidneys were removed. The expression levels of renal transporters and platinum (Pt) accumulation were analyzed. The serum creatinine level was significantly increased by CDDP administration and treatment with Mg significantly ameliorated such elevation. The expressions of the renal organic cation transporter 2 (rOct2) and renal multidrug and toxin extrusion protein 1 (rMate1) were downregulated and upregulated, respectively following co-administration with Mg, which significantly reduced the renal Pt accumulation in the 2.5 mg/kg CDDP-treated group. Moreover, Mg dose-dependently downregulated rOct2, not affecting rMate expression, resulting in the attenuation of CIN. Mg co-administration protected the downregulation of the transient receptor potential subfamily Melastatin 6 (rTrpm6), but not the epidermal growth factor (rEgf), as a result, Mg co-injection attenuated CDDP-induced hypomagnesemia. In conclusion, Mg co-administration reduced Pt accumulation by regulating the expression of the renal transporters, rOct2 and rMate1 and, thereby, attenuated CIN.

1. Introduction

Nephrotoxicity is one of the major, most serious, and dose-limiting toxicities caused by cisplatin (cis-dichloro-diammine platinum, CDDP), which is a widely used chemotherapeutic agent for the lung, gastric, head and neck, ovarian, and urological malignancies (Go and Adjei, 1999). CDDP-induced nephrotoxicity (CIN) is recognized to be cumulative, dose-related, and usually reversible, occurring in 30–40% of patients administered with CDDP (Pabla and Dong, 2008; Miller et al., 2010; Yoshida et al., 2014). CIN has been suggested to especially affect the S3 segment of the proximal tubule located in the outer medulla, and the thick ascending limb of the loop of Henle (Dobyan et al., 1980). The mechanism of CIN induction is thought to be DNA damage, oxidative stress, mitochondrial dysfunction, inhibition of protein synthesis, and increased tumor necrosis factor (TNF) family (Tsuruya et al., 2003; Brady et al., 1990; Park et al., 2002).

Hypomagnesemia has been reported to occur in approximately 90% of the patients administered with CDDP (Lajer and Daugaard, 1999), and damage to the renal tubular cells and Ca^{2+}/Mg^{2+} sensing receptor causes CDDP-induced hypomagnesemia and hypocalcemia (Lajer and Daugaard, 1999; Vickers et al., 2004). It has also been reported that the transient receptor potential subfamily Melastatin 6 (rTrpm6), which is present on the distal convoluted tubule (DCT) and the epidermal growth factor (rEgf) interact and have important roles in Mg reabsorption in the DCT (Ledeganck et al., 2013). Furthermore, renal mRNA expression of rTrpm6 and rEgf significantly decreased after 2.5 mg/kg

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http://dx.doi.org/10.1016/j.ejphar.2017.05.034
Received 25 January 2017; Received in revised form 7 May 2017; Accepted 17 May 2017
Available online 18 May 2017
0014-2999/ © 2017 Published by Elsevier B.V.

CDDP administration, leading to increased Mg excretion (Ledeganck et al., 2013). Mg is the second most common intracellular cation in the human body (Tong and Rude, 2005) and acts as a cofactor for approximately 300 cellular enzymes that modulate cellular energy metabolism involving adenosine triphosphate (ATP), muscle Na^+/K^+-pump activity, calcium channel activity, and stabilization of membrane structures, as well as RNA/DNA polymerase, which is responsible for mRNA translation, transcription, and replication of DNA (Wacker and Parisi, 1968; Dørup et al., 1988; Reinhart, 1991; Mildvan and Loeb, 1979). Yokoo et al. reported that the organic cation transporter 2 (rOct2), which is predominantly expressed in the basolateral membranes of proximal tubules and is responsible for CDDP accumulation into the S3 segment, is expressed Mg-dependently and hypomagnesemia causes upregulation of rOct2 (Yokoo et al., 2009). They also suggested that the expression level of multidrug and toxin extrusion protein 1 (rMate1), which transports CDDP from the proximal tubule into the urine, tended to decrease in hypomagnesemic rats (Yokoo et al., 2009).

Clinically, Willox et al. and Bodnar et al. reported that oral and intravenous Mg supplementation ameliorates CDDP-induced serum creatinine or urinary N-acetyl-β-D-glucosaminidase (NAG) elevation (Willox et al., 1986; Bodnar et al., 2008). We have also reported that intravenous Mg premedication alone prevents the incidence of CIN and ameliorates its severity (Saito et al., 2017). However, the mechanism underlying renal protection by Mg co-administration with CDDP is not fully understood. Therefore, this present study was conducted to determine how Mg co-administration attenuates CIN, focusing on the renal transporter expression level.

2. Materials and methods

2.1. Chemicals

CDDP was purchased from Sigma-Aldrich Chemical Corp., (St. Louis, USA). The rOct2- and rMate1-specific primary antibodies were from Santa Cruz Biotechnology (Santa Cruz, USA), rTrpm6 was from GeneTex (California, USA), and rActin was from Merck Millipore (Billerica, USA). Creatinine was measured using a kit (L type Wako creatinine F or CRE·M) purchased from Wako (Osaka, Japan). Serum Mg was determined using a Metallo Assay Mg LS kit from Metallogenics (Chiba, Japan). All other chemicals and reagents were commercially available and of the highest purity possible.

2.2. Animals

Male Wistar rats (7-weeks-old) weighing 210–230 g were obtained from JLA (Tokyo, Japan). All animals were housed in a standard animal maintenance facility in a temperature (23 °C) and moisture (60 ± 10%) controlled room with 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.

2.3. Experimental design

CDDP 2.5 or 5 mg/kg (1 or 2 mg/ml, respectively) or saline was administered intraperitoneally as a therapeutic agent while Mg sulfate 20 mg/kg (8 mg/ml) or 40 mg/kg (16 mg/ml), or saline was administered intraperitoneally as a prophylactic agent 4 h prior to treatment with the therapeutic agent. The interval between the administration of the therapeutic and prophylactic agents was set at 4 h, which was the shortest time during which the prophylactic agent did not affect the blood levels of the therapeutic agent in our preliminary study. Thirty-six rats were divided into six groups and treated as follows:

(1) Control group, saline prophylactically and therapeutically.
(2) Mg group, 40 mg/kg Mg prophylactically and saline therapeutically.
(3) 2.5 mg/kg CDDP group, saline prophylactically and 2.5 mg/kg CDDP therapeutically.
(4) 2.5 mg/kg CDDP-Mg group, 40 mg/kg Mg prophylactically and 2.5 mg/kg CDDP therapeutically.
(5) 5 mg/kg CDDP group, saline prophylactically and 5 mg/kg CDDP therapeutically.
(6) 2.5 mg/kg CDDP-half Mg group, 20 mg/kg Mg prophylactically and 2.5 mg/kg CDDP therapeutically.

Blood samples were obtained from the right jugular vein at baseline (before prophylactic agent administration) and 120 and 168 h after the therapeutic agent was injected. The body weight of the animals was measured following the same timing as the blood sampling. After blood collection at 168 h, the rats were anesthetized with ether, euthanized, and the kidneys were collected immediately and the tissue was stored with the serum samples at −80 °C until analyzed.

2.4. Western blot analysis

The expression levels of rOct2, rMate1 and rTrpm6 were assessed using western blot analysis. The cortex from the kidney was sliced and total protein extracts were prepared by homogenizing in lysis buffer containing 1.0% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), and 4.5 M urea. The homogenate was sonicated for 15 min at 4 °C, centrifuged at 12100×*g* for 15 min at 4 °C, and then the samples were denatured at 100 °C for 3 min in a loading buffer containing 0.1 M Tris-hydrochloride, 4% SDS, 10% 2-mercaptoethanol, 20% glycerol, 0.004% bromophenol blue (BPB). Then, the samples were separated using 10% for rOct2 and rMate1, 7.5% for rTrpm6 SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto polyvinylidene difluoride membranes (Bio-Rad, Tokyo, Japan) by semidry electroblotting at 15 V for 90 min. The membranes were blocked with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS/T) and 1% non-fat dry milk for 1 h at room temperature. After washing with PBS/T, the membranes were incubated overnight with primary antibodies against rOct2 (1:200), rMate1 (1:100), and rTrpm6 (1:200), washed thrice with PBS/T for 10 min each time, incubated for 1 h with a goat anti-rabbit IgG-horseradish peroxidase (HRP) secondary antibody (Santa Cruz Biotechnology, Santa Cruz, USA) against rOct2, rMate1 and rTrpm6 (1:2000,1:200 and 1:2000, respectively), and then washed thrice with PBS/T for 10 min each time. After washing with PBS/T, the bound antibodies were detected using the Image Quant LAS 4000 (GE Healthcare UK Ltd., Amersham Place, UK) by enhanced chemiluminescence. The protein concentration in the clear supernatant was determined using the method of Lowry et al. (1951).

2.5. Isolation of total RNA and reverse transcription-polymerase chain reaction

2.5.1. (RT-PCR) analysis

Total RNA was extracted from the homogenized kidney tissue using an ISOGEN II (Nippon Gene, Tokyo, Japan) kit according to the manufacturer's protocol. RNA concentration was determined by measuring the absorbance at a wavelength of 260 nm. One microgram of total RNA was used to prepare complementary DNA by reverse transcription using ReverTra Ace (Toyobo, Osaka, Japan). The levels of mRNAs for tissue Inhibitor of Metalloproteinase-1 (rTimp-1), rTrpm6 and rEgf were measured using real-time polymerase chain reaction (qPCR). Table 1 shows the primer sequences used for the PCR amplification and the products were normalized to amplified rActin, the internal reference gene. Standard curves were constructed for each target and housekeeping gene, as well as between the threshold cycles (Ct) and the log 10 (copy numbers) using the Applied Biosystems (Agilent Technologies, Santa Clara, USA) sequence detection system

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