Effects of cadmium on the gene expression of SLC39A1 coding for ZIP1 protein

Jin-Yong Lee¹, Maki Tokumoto¹, Yasuyuki Fujiwara¹,², Moo-Yeol Lee³ and Masahiko Satoh¹

¹Laboratory of Pharmaceutical Health Sciences, School of Pharmacy, Aichi Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan
²Department of Environmental Health, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan
³College of Pharmacy, Dongguk University, Goyang, Gyeonggi-do 410-820, Republic of Korea

(Received November 17, 2014; Accepted November 19, 2014)

ABSTRACT — Cadmium (Cd) is a toxic heavy metal, particularly in the kidney. Zinc transporters have been reported to be responsible for the absorption of Cd in the kidney. Interestingly, we previously found in a DNA microarray that exposure to Cd suppressed the expression of the gene coding for the zinc transporter ZIP1 (SLC39A1) in HK-2 human kidney proximal tubular cells. In this study, we validated by real-time RT-PCR that SLC39A1 gene expression was indeed decreased upon treatment with 40 μM Cd. We also demonstrated that knockdown of SLC39A1 by siRNA transfection conferred resistance to Cd in HK-2 cells. Together, this suggests that gene suppression of SLC39A1 by Cd is involved in the defense mechanism against the Cd toxicity in HK-2 cells.

Key words: Cadmium, HK-2 cells, SLC39A1

INTRODUCTION

Cadmium (Cd) is a toxic heavy metal that causes severe clinical symptoms in various tissues including the kidney (Järup and Akesson, 2009). Owing to its long biological half-life (10-30 years), Cd can accumulate in the kidney (Järup, 2002; Järup and Akesson, 2009). Accumulated Cd in the kidney of mice has been reported to alter gene expression (Tokumoto et al., 2013). Cd-induced renal toxicity is initialized by proximal tubular damage (Järup and Akesson, 2009). However, the underlying mechanism of Cd toxicity remains unclear. Recently, we have conducted DNA microarray analysis using human proximal tubular cells (HK-2 cells) treated with 40 μM Cd for 3 hr (Lee et al., 2013). In the Cd-treated HK-2 cells, expression of 30 genes was elevated more than 2.0-fold, and expression of 21 genes was reduced less than 0.5-fold. Among the down-regulated genes, the SLC39A1 gene coding for Zinc transporter (ZIP1) was included. This was of particular interest because zinc transporters, such as ZIP8 and ZIP14, have been shown to be involved in the absorption of Cd in the kidney (Fujishiro et al., 2012). In this study, we examined the involvement of ZIP1 in Cd toxicity in human proximal tubular cells.

MATERIALS AND METHODS

Cell culture

HK-2 cells were cultured in Dulbecco’s Modified Eagle’s Medium/Ham’s Nutrient Mixture F-12 (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 25 U/mL penicillin, 25 μg/mL streptomycin, 1% Insulin-Transferrin-Selenium-X (Gibco), 10 ng/mL EGF and 5 ng/mL hydrocortisone at 37°C in a humidified incubator containing 5% CO₂.

Real time RT-PCR

HK-2 cells were transferred into a 60-mm tissue culture dish (Falcon, Franklin Lakes, NJ, USA) at a density of 2.0 × 10⁴ cells/cm² and cultured until confluent. Then, the culture medium was discarded and the cells were treated with 40 μM cadmium chloride (CdCl₂; Wako Pure Chemical Industries, Osaka, Japan) in serum-free culture medium for 6 hr. Cd-treated HK-2 cells were washed twice with ice-cold phosphate buffered saline (PBS; Nissui, Tokyo, Japan). Total RNA was extracted with the Quick Gene RNA cultured cell kit 5 (Fujifilm, Tokyo, Japan), according to the manufacturer’s protocol.
Total RNA was incubated with the PrimeScript reverse transcription (RT) Reagent Kit (Perfect Real Time) (Takara Bio, Shiga, Japan) to generate cDNA. Real-time PCR was performed with SYBR Premix Ex TaqII (Perfect Real Time) (Takara Bio) and the Thermal Cycler Dice Real Time System (Takara Bio). PCR conditions were as follows: 10 sec hot-start at 95°C followed by 40 cycles of 5 sec at 95°C and 30 sec at 60°C. Gene expression was normalized to GAPDH mRNA levels. The oligonucleotide sequences of the primers were as follows: sense, 5'-GCACCGTCAAGGCTGAGAAC-3', and antisense, 5'-TTGGTAGAGACGCCAGTGG-3', for the human GAPDH gene; sense, 5'-GCCAGGAGCTAACCATGAAG-3', and antisense, 5'-ATGGCCAGGATGAACTCTTG-3', for the human SLC39A1 gene.

siRNA transfection

Silencer Select Pre-designed siRNA were purchased from Ambion (Grand Island, NY, USA). The ID of siRNA was as follows: s25947 (Silencer® Select Pre-designed siRNA), for human SLC39A1. siRNA transfection was performed using Lipofectamine RNAiMAX (Invitrogen, Grand Island, NY, USA). After the siRNA mixture was incubated for 15 min with Lipofectamine RNAiMAX and Opti-MEM® I Reduced Serum Medium (Gibco), HK-2 cells were transfected with the siRNA mixture for 24 hr.

Cell viability

HK-2 cells were transferred to a 96-well tissue culture plate (Falcon) at a density of 2.0 × 10^4 cells/cm² for 24 hr and then the siRNA mixture (1 nM siRNA, 0.2% [v/v] Lipofectamine RNAiMAX, 10% [v/v] Opti-MEM® I Reduced Serum Medium) was added. After 24 hr, the cells were incubated with CdCl₂ in a serum-free culture medium for 24 hr. After treatment, the serum-free medium containing CdCl₂ was replaced with fresh growth medium containing 10% (v/v) Alamar Blue (Invitrogen) and incubated for 4 hr at 37°C. Fluorescence was measured at excitation wavelength of 540 nm and an emission wavelength of 595 nm.

Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA). When the F value was significant (P < 0.05), Bonferroni’s multiple t-test was performed for post-hoc comparison (P < 0.05).

RESULTS AND DISCUSSION

In our previous study, we found that the gene expression of SLC39A1, which codes for the ZIP1 protein, decreased upon treatment with 40 μM Cd for 3 hr (Lee et al., 2013). Therefore, we examined the mRNA levels of SLC39A1 in HK-2 cells treated with 40 μM Cd for various time points. Indeed, the mRNA level of SLC39A1 was significantly decreased upon 3 hr and 6 hr treatment with Cd (Fig. 1). Next, we transfected double-stranded siRNA against SLC39A1 into HK-2 cells. As expected, SLC39A1 mRNA levels in the cells transfected with SLC39A1 siRNA was significantly decreased compared with control cells (Fig. 2A). To investigate the role of SLC39A1 in Cd toxicity, we compared the viability of SLC39A1 siRNA transfected cells with that of control cells. HK-2 cells transfected with SLC39A1 siRNA were slightly resistant to Cd treatment compared with control cells (Fig. 2B). These results suggest that protein coded by SLC39A1, ZIP1, may be involved in the promotion of Cd toxicity. Considering Cd decreased the gene expression of SLC39A1 in HK-2 cells, one would expect less ZIP1 to be present in the cell membrane after acute exposure to Cd. Thus, it seems unlikely that ZIP1’s role in Cd toxicity is due to the uptake of Cd into HK-2 cells. ZIP1 protein is distributed not only on the cell membrane but also on intracellular vesicles (Michalczyk and Ackland, 2012; Jeong and Eide, 2013). Therefore, an intracellular...
lar transporting function of ZIP1 may be responsible for the observed Cd toxicity. Alternatively, the decrease in \textit{SLC39A1} may be the result of a negative feedback loop that prevents further uptake of Cd by ZIP1. Further investigation into the role of ZIP1 in Cd toxicity should help decipher the mechanism of Cd toxicity.

**ACKNOWLEDGMENT**

This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan.

**Conflict of interest**—— The authors declare that there is no conflict of interest.

**REFERENCES**


Fig. 2. Effects of knockdown of \textit{SLC39A1} on Cd toxicity in HK-2 cells. (A) Knockdown efficiency of \textit{SLC39A1} in HK-2 cells following \textit{SLC39A1} siRNA treatment. \textit{SLC39A1} siRNA was added to HK-2 cells and incubated for 24 hr. mRNA levels were examined using real-time RT-PCR. mRNA levels were normalized to \textit{GAPDH}. Values are the mean ± S.D. (\textit{n} = 3). (B) Cell viability of HK-2 cells transfected with \textit{SLC39A1} siRNA or control siRNA after treatment with cadmium chloride for 24 hr using the Alamar Blue assay. Values are the mean ± S.D. (\textit{n} = 3). *Significantly different from the corresponding control siRNA group, \textit{P} < 0.05.