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Letter

Effect of chronic cadmium exposure on the gene expression of Birc family in the mouse kidney and liver

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ABSTRACT — Cadmium (Cd) is a toxic metal that can cause renal proximal tubular cell damage. Our previous research demonstrated that Cd induces apoptosis by suppressing the *BIRC3* gene expression in human proximal tubular cells (HK-2 cells). BIRC3, a member of BIRC family, inhibits apoptosis by suppressing caspase activity. Cd has been shown to induce caspase-3 activation through the suppression of *BIRC3* expression in HK-2 cells. In this study, we examined Birc family gene expression in the kidney and liver of mice exposed to Cd for 67 weeks. Cd exposure decreased the expression of *Birc3* in the kidney but increased *Birc3* expression in the liver of mice. In our previous *in vitro* study, Cd decreased *BIRC3* expression predominantly in proximal tubular cells. The present findings strongly indicate that the decrease in *BIRC3* gene expression is implicated in the induction of apoptosis by Cd in proximal tubules.

Key words: Cadmium, Chronic exposure, Birc3, Kidney

INTRODUCTION

Cadmium (Cd) is used in industrial applications such as steel material plating, batteries, and pigments (Nordberg *et al*., 2014). However, Cd enters the environment from industrial waste, resulting in human exposure as well as uptake by various foods (Satarug *et al*., 2010). Cd can be found in high doses in seafood, vegetables, cereals, animal products, and even in chocolate (Fowler *et al*., 2014). Cd exerts toxic effects in various tissues such as the kidney, liver, testis, lung, and bone (Järup *et al*., 1998; Satoh *et al*., 2002; Nordberg *et al*., 2014). Because of its long biological half-life (10-30 years), high concentrations of Cd can accumulate in the human body over time, especially in the kidney (Järup *et al*., 1998; Järup *et al*., 2009; Nordberg *et al*., 2014). Renal proximal tubular cells are thought to be the main target of Cd kidney toxicity (Järup *et al*., 1998; Järup *et al*., 2009; Fujiwara *et al*., 2012; Nordberg *et al*., 2014). However, the target factor of Cd toxicity remains unclear.

Our previous research demonstrated that Cd inhibited ARNT transcription factor activity in human proximal tubular cells (HK-2 cells) (Lee *et al*., 2017). Furthermore, we showed that Cd decreased the gene expression of *BIRC3*, an apoptosis-inhibiting factor, through the suppression of ARNT activity. *BIRC3* is one of eight members (seven members in mice) of the BIRC family (LaCasse *et al*., 2008). Cd has been shown to suppress only *BIRC3* expression in HK-2 cells; moreover, methylmercury and arsenic did not suppress *BIRC3* (*Birc3*) gene expression in human neuroblastoma and mouse hepatocytes, respectively (Lee *et al*., 2017). In this study, we examined the expression of Birc family genes in the kidney and liver of mice chronically exposed to Cd.

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MATERIALS AND METHODS

Animals and treatment

All animal experiments were undertaken in accordance with the Regulations on Animal Experimentation at the School of Pharmacy, Aichi Gakuin University (Nagoya, Japan). All procedures for the maintenance and use of mice were approved by the Animal Care and Use Committee of the School of Pharmacy, Aichi Gakuin University.

Wild-type 129/Sv mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and bred routinely in the laboratory animal facility of Aichi Gakuin University. Five-week-old female mice were housed in a ventilated animal room at 23°C ± 1°C with relative humidity and a 12-hr light/dark cycle.

Mice were randomly assigned to control or experimental groups. Control mice were fed standard laboratory chow, while experimental mice were fed chow containing 300 ppm Cd (Oriental-BioService, Kyoto, Japan). All mice had unlimited access to tap water. After 67 weeks of Cd exposure, the kidney and liver were removed from each mouse under ether anesthesia.

Measurement of Cd concentration

Tissues were digested using nitric acid and hydrogen peroxide before inorganic residues were dissolved in ultrapure water and metal analysis was carried out using an atomic absorption spectrometer (200 series AA; Agilent Technologies, Santa Clara, CA, USA).

Real time RT-PCR

Total RNA was extracted from mouse tissue using a QuickGene RNA Tissue Kit S (Fujifilm, Tokyo, Japan) according to the manufacturer's instructions. cDNA was generated from total RNA using a PrimeScript™ RT Reagent Kit (Perfect Real Time) (TaKaRa Bio, Shiga, Japan). Real-time PCR was performed using SYBR Premix Ex Taq™ II (TaKaRa Bio) and a Thermal Cycler Dice real-time system (TaKaRa Bio). PCR conditions were as follows: 10 sec of 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 *ß-actin* mRNA levels. The oligonucleotide sequences of the primers were as follows: sense, 5′-CATGTGTGTGGAGGGTGAAG-3′ and antisense, 5′-TTTAACAGGGGACAGCATCC-3′ for the mouse *Birc1* gene; sense, 5′-GCATTTTCCCAACTGTCCAT-3′ and antisense, 5′-ATTCGAGCTGCATGTGTCTG-3′ for the mouse *Birc2* gene; sense, 5′-CAACAGATCTGGCAAAAGCA-3′ and antisense, 5′-TTGCTCAATTTTCCACCACA-3′ for the mouse *Birc3* gene; sense, 5′-TGGGGTTCAGTTTCAAGGAC-3′ and antisense, 5′-TGCAACCAGAACCTCAAGTG-3′ for the mouse *Birc4* gene; sense, 5′-GTTGCGCTTTCCTTTCTGTC-3′ and antisense, 5′-TCTCCGCAGTTTCCTCAAAT-3′ for the mouse *Birc5* gene; sense, 5′-TGACGCTTTCAACCTCACTG-3′ and antisense, 5′-GTGTCCGCTGGACCAGTTAT-3′ for the mouse *Birc6* gene; sense, 5′-TGGCCTCCTTCTATGACTGG-3′ and antisense, 5′-ACCTCACCTTGTCCTGATGG-3′ for the mouse *Birc7* gene; and sense, 5′-CCTAAGGCCAACCGTGAAAA-3′ and antisense, 5′-AGCCATACAGGGACAGCACA-3′ for the mouse *ß-actin* gene.

Statistical analyses

Statistical analyses were undertaken using Student's *t*-test for comparisons between two factors ($P < 0.05$).

RESULTS AND DISCUSSION

Body weights of mice were monitored upon exposure to Cd for 67 weeks, and were found to be significantly lower in Cd-exposed mice than in non-exposed mice (Lee *et al*., 2016). Accumulated Cd levels of 189.55 ± 12.85 μg/g have previously been found in the kidney of mice (Sarma *et al*., in press), while levels of 287.74 ± 66.43 μg/g have been found in the liver.

To determine the renal toxicity of Cd, *N*-acetyl-ß-*D*-glucosaminidase (NAG) activity in the urine and BUN value in the serum were examined. The NAG activity in the urine and blood urea nitrogen (BUN) values in the serum of Cd-exposed mice were slightly increased (Sarma *et al*., in press). Therefore, Cd caused mild renal toxicity following long-term exposure in mice. To determine the hepatotoxicity of Cd, serum glutamic oxaloacetic transminase (GOT) and glutamate pyruvate transminase (GPT) activity was examined and found to be slightly increased upon Cd exposure (Lee *et al*., 2016). Therefore, long-term Cd exposure caused mild toxicity in the kidney and liver.

In the present study, Cd caused a decrease in *Birc3* mRNA levels in the kidney of mice (Fig. 1C). However, Cd exposure did not affect the kidney mRNA levels of *Birc1*, *Birc2*, *Birc4*, and *Birc7* (Figs. 1A, 1B, 1D, and 1G). Further, mRNA level of *Birc5* was increased while *Birc6* was decreased (Figs. 1E and 1F). In the liver, Cd exposure increased the expression of *Birc1* and *Birc3* mRNA (Figs. 2A and 2C) but decreased the expression of *Birc4* and *Birc6* (Figs. 2D and 2F). The mRNA levels of *Birc2*, *Birc5*, and *Birc7* were not affected by Cd exposure in the liver (Figs. 2B, 2E, and 2G). These results suggest that Cd may suppress *BIRC3* gene expression specifical-

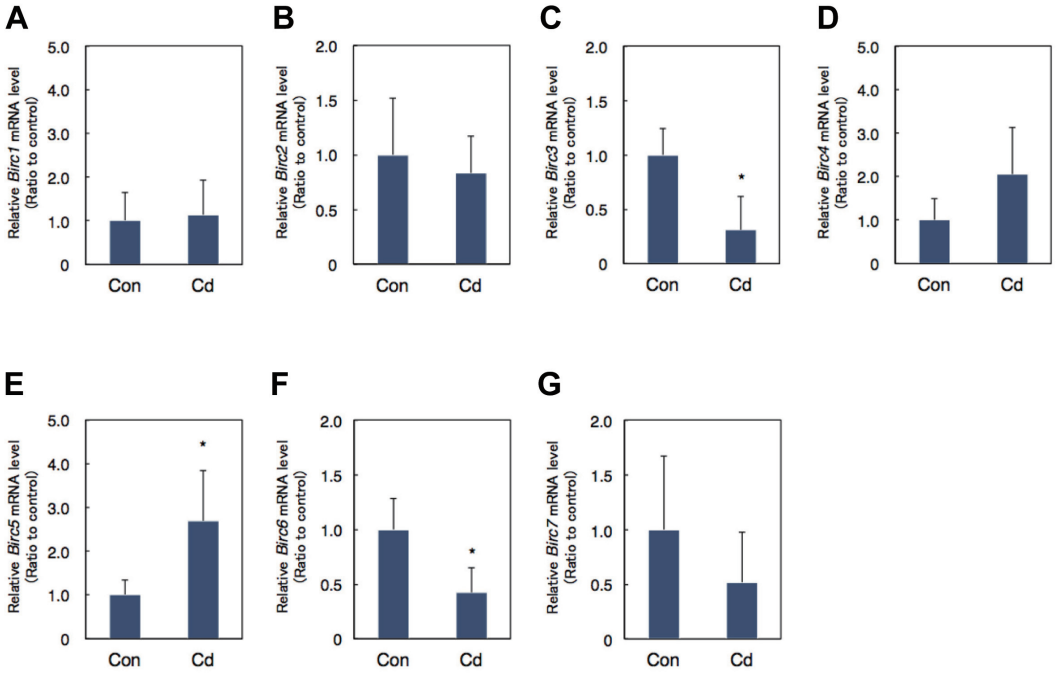

Fig. 1. Effect of Cd on the mRNA levels of Birc family genes in the kidney of mice exposed to Cd for 67 weeks. The mRNA levels were determined using real-time RT-PCR. (**A**) *Birc1*, (**B**) *Birc2*, (**C**) *Birc3*, (**D**) *Birc4*, (**E**) *Birc5*, (**F**), *Birc6* and (**G**) *Birc7*. mRNA levels were normalized to *β-actin*. Values are the means ± S.D. (n = 4-5). *Significantly different from the control group, $P < 0.05$.

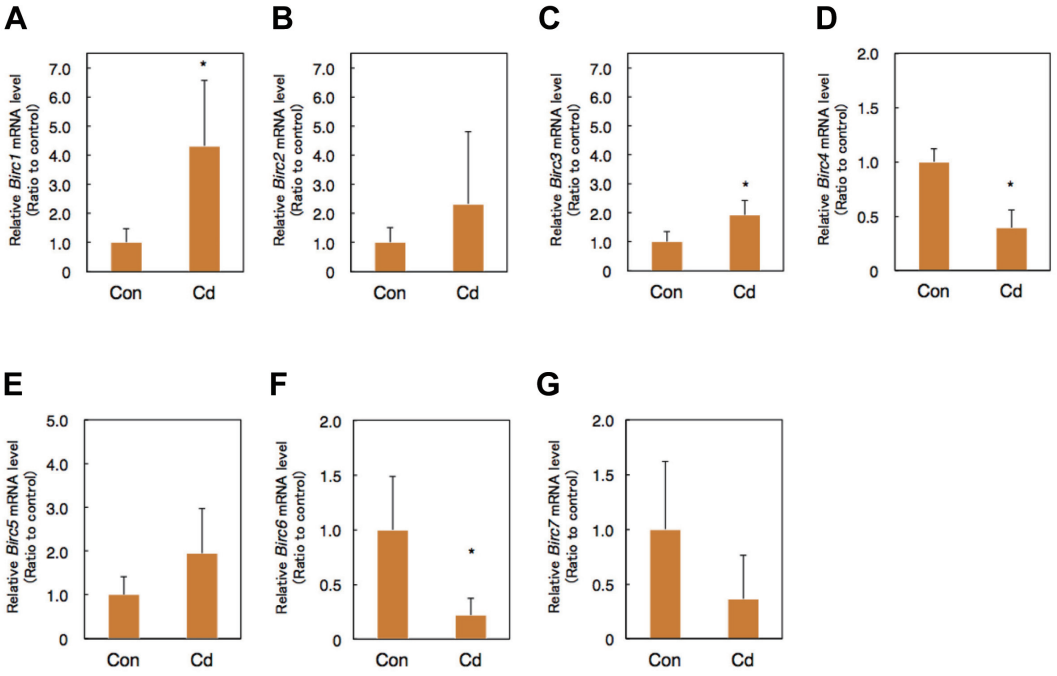

Fig. 2. Effect of Cd on the mRNA levels of Birc family genes in the liver of mice exposed to Cd for 67 weeks. The mRNA levels were determined using real-time RT-PCR. (**A**) *Birc1*, (**B**) *Birc2*, (**C**) *Birc3*, (**D**) *Birc4*, (**E**) *Birc5*, (**F**) *Birc6*, and (**G**) *Birc7*. mRNA levels were normalized to *β-actin*. Values are the means ± S.D. (n = 4-5). *Significantly different from the control group, $P < 0.05$.

ly in the kidney.

Our previous research indicated that Cd-induced suppression of *BIRC3* gene expression increased the level of the active form of caspase-3 (Lee *et al*., 2017). However, recent studies have shown that BIRC family members are functionally non-equivalent and regulate caspase activities *via* distinct mechanisms (Choi *et al*., 2009; Eckelman and Salvesen, 2006; Tenev *et al*., 2005). Moreover, BIRC5 protein, also known as Survivin, plays a key role in cell survival (Chen *et al*., 2014). In this study, *Birc5* mRNA expression in the kidney was increased by Cd exposure. Therefore, the BIRC family may function as protective factors against Cd toxicity.

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

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