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The potential protective role of lysophospholipid mediators in nephrotoxicity induced by chronically exposed cadmium

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ABSTRACT

Cadmium is a hazardous metal whose chronic exposure induces renal failure due to fibrosis, but the mechanisms are not well known. In this study we analyzed the molecular species of lysophosphatidic acid (LPA) and related phospholipids, together with their metabolic enzyme activity, in plasma from Wistar rats exposed up to 300 ppm Cd^{2+} in drinking water for 114 days. Exposure of 300 ppm Cd^{2+} for 114 days enhanced autotoxin (ATX)/lysophospholipase D activity, but significantly lowered the total levels of LPA and lysophosphatidylethanolamine. Interestingly, the total level of sphingosine-1-phosphate (S1P) was elevated dose-dependently by Cd^{2+}. Cultured rat kidney-derived interstitial fibroblast cells, NRK49F cells and proximal epithelial cells, NRK52E cells, were both responsive to the protective action of LPA or S1P against Cd^{2+} toxicity. The former cell expresses ATX RNA. In conclusion, the elevation of LPA-producing enzyme activity and S1P concentrations in plasma after exposure of rats to Cd^{2+} would protect from the renal toxicity of Cd^{2+}.

1. Introduction

Cadmium (Cd) is a heavy metal that accumulates in human kidney, liver, lung, testes, bone and nerve tissue, damaging them (Brzoska and Moniuszko-Jakoniuk, 2005; Kundomal et al., 1986; Sato et al., 1978; Suzuki, 1980). The level of Cd^{2+} was highest in the kidney of rats after oral chronic exposed to Cd^{2+} (Saygi et al., 1991). Once Cd^{2+} is taken into the circulation mainly in a form bound to albumin, it becomes incorporated into the liver. Chronic Cd^{2+} exposure induces expression of metallothionein, a low molecular weight cysteine-rich protein (6–7 kDa). The Cd-metallothionein is released into the bloodstream (Dorian et al., 1995; Dudley et al., 1985, 1982), ultrafiltered by glomeruli like free Cd^{2+}, and then reabsorbed by transporters such as divalent metal transporter 1 (Abouhamed et al., 2007; Bridges and Zalups, 2005), megalin, and cubilin (Christensen and Nielsen, 2007). Cd^{2+} is predominantly located in the S1 and S2 segments of kidneys, and induces renal tubular dysfunction (Nogawa et al., 1975), proteinuria in cadmium-exposed workers (Friberg, 1950), and renal cancer (Il'yasova and Schwartz, 2005; Pesch et al., 2000).

There are many reports of various studies of Cd^{2+}-induced kidney impairment. In toxicological studies with Cd^{2+} using an experimental animal, especially rat and mouse, Cd^{2+} was administered intraperitoneally, orally, subcutaneously, or intravenously, for various time periods. In these animal models, histological impairment of the kidney eventually became extensive (Aughey et al., 1984; Jihen el et al., 2008; Tripathi and Srivastav, 2011). For example, after $CdCl_2$ was administered orally to rats for eight weeks, shrinkage of glomeruli, tubular dilatation, and hypertrophy of tubular epithelium were observed (Tripathi and Srivastav, 2011). Tubular necrosis and glomerular widening were observed in the kidneys of rats given Cd^{2+} in drinking water for 35 days (Jihen el et al., 2008). Generally, chronic intake of Cd^{2+} is known to induce renal fibrosis derived from an epithelial-to-mesenchymal transition.

Abbreviations: αSMA, α-smooth muscle actin; ATX, autotaxin or ectonucleotide pyrophosphatase/phosphodiesterase 2; Cd, cadmium; $CdCl_2$, cadmium chloride; LC–MS/MS, chromatography–tandem mass spectrometry; DMEM, dulbecco's modified Eagle's medium; FCS, fetal calf serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LPP, lipid phosphate phosphatase; LPA_1, LPA_1 receptors; LPA, ysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; LPI, lysophosphatidylinositol; LPS, lysophosphatidylserine; lysoPLD, lysophospholipase D; $S1P_1$, S1P1 receptor; SPHK1, sphingosine kinase 1; SPHK2, sphingosine kinase 2; S1P, sphingosine-1-phosphate; UUO, unilateral ureteral obstruction.

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Some mechanisms of kidney toxicity produced by Cd^{2+} have been proposed, including oxidative DNA damage induced by reactive oxygen species (Liu et al., 2009), p53-triggered renal obstruction, inhibition of Na^+-dependent glucose transporter (Blumenthal et al., 1990), and inhibition of Na^+ K^+-ATPase (Van Kerkhove et al., 2010).

Kidney function is impaired by multiple factors. Unilateral ureteral obstruction (UUO) in mice and rats is used as an experimental model of renal failure (Chevalier et al., 2009). Pradère et al. showed that the level of lysophosphatidic acid (LPA) released from the ligated side of kidney explants in mice was increased about 3-fold compared with that of sham-operated mice, and that this increase was responsible for renal interstitial fibrosis via LPA_1 receptors (Pradère et al., 2007). Like sphingosine-1-phosphate (S1P), LPA behaves as a lipid mediator though several G-protein-coupled receptors, exerting diverse physiological activities, involving cell proliferation, prevention of apoptosis, carcinogenesis, vasoactivity, and angiogenesis (Durieux and Lynch, 1993; Jalink et al., 1994; Obinata and Hla, 2012; Tokumura, 1995). The lysophospholipase D (lysoPLD) activity of autotaxin (ATX) produces LPA from several lysophospholipids (LPLs). S1P is mainly produced by phosphorylation of sphingosine by sphingosine kinases (Obinata and Hla, 2012). Recently, we detected a high level of LPA in renal effluent of rats after UUO by liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Tsutsumi et al., 2011). Previously, a single peritoneal injection of Cd^{2+} was reported to alter levels of major phospholipids including lysophosphatidylcholine (LPC) in rat liver and brain mitochondria (Modi and Katyare 2009a,b), and LPA_1 is involved in dysplastic lesion on ventral prostate by $CdCl_2$ (Arriazu et al., 2013). However, there was no report focusing effects of Cd^{2+} on body level and renal function of lysophopsholipid (LPL) mediators such as LPA.

In this study, we examined whether plasma levels of LPA, S1P, and related LPL are affected by exposure of rats to Cd^{2+}. Furthermore, we examined whether LPA and S1P attenuate the cell death of rat renal cells induced by Cd^{2+}.

2. Materials and methods

2.1. Materials

Cadmium chloride ($CdCl_2{\cdot}2.5\ H_2O$) was purchased from Wako Pure Chemical (Osaka, Japan). 1-Heptadecanoyl (17:0)-LPC and 17:1-lysophosphatidylinositol (LPI) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). 17:0-LPA was prepared from 17:0-LPC using phospholipase D from *Streptomyces chromofuscus* as described previously (Tokumura et al., 1994). Standard phosphatidylethanolamine (LPE), 17:0-lysophosphatidylserine (LPS) and 17:0-lysophosphatidylinositol (LPI) were prepared by incubation of 17:0/17:0-phosphatidylethanolamine, 17:0/17:0-phosphatidylserine and 17:0/17:0-phosphatidylglyceol (500 μg phosphorus, Avanti Polar Lipids), respectively, with pancreatic phospholipase A_2 (100 units/ml) at 37 °C for 3 h, followed by lipid extraction by the method of Bligh and Dyer under acidic conditions and TLC purification. Both 17:0-LPS and 17:0-LPG were prepared by incubation of 17:0/17:0-phosphatidylserine and 17:0/17:0-phosphatidylglyceol (500 μg phosphorus, Avanti Polar Lipids), respectively, with pancreatic phospholipase A_2 (100 units/ml) at 37 °C for 3 h, followed by lipid extraction by the method of Bligh and Dyer under acidic conditions and TLC purification. Phospholipid standards were obtained from Funakoshi Co. (Tokyo, Japan). They included egg yolk phosphatidylcholine, 1-palmitoyl LPC, and 1-palmitoyl LPA. Dulbecco's modified Eagle's medium (DMEM) was purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA).

2.2. Animal experiments

Male Wistar/ST rats 6–7 weeks old (Japan SLC, Shizuoka, Japan) were randomly divided into two groups (9 or 10 animals), and given drinking water with 0 or 300 ppm Cd^{2+} for 114 days. The breeding room was kept at 20–25 °C with a light-dark cycle of 12 h each. Rats were given free access to food and water throughout the experimental period. All rats were handled in accordance with the principles and guidelines of the Experimental Animal Committee of Kyushu University of Health and Welfare.

2.3. Preparation of plasma

Blood was obtained by puncturing the heart after killing the rats and mixed with heparin (500 units/ml) or EDTA (3 mg/ml). Plasma was prepared from the heparinized and EDTA-anticoagulated blood samples by centrifugation at 1200*g* for 20 min at 4 °C, and used for measurement of lysoPLD activity and quantifications of LPA, LPC, LPI, LPE, LPG, LPS, and S1P, respectively. In order to prevent the degradation of LPA by enzymes such as lysoPLD during preparation of plasma, EDTA was used as an anticoagulant for accurate assessment of the in vivo circulating level of LPA.

2.4. Histological analysis

Kidneys were quickly removed, and slices were fixed with 10% formaldehyde and then embedded in paraffin. Degrees of renal tissue injury were evaluated after staining with hematoxylin/eosin.

2.5. Lipid extraction

Lipids were extracted from plasma of EDTA-anticoagulated blood by the method of Bligh and Dyer after adjusting the pH of the aqueous phase to 8–9 with 20% ammonium hydroxide (Bligh and Dyer, 1959). To the lipid extract were added 17:0-LPC, 17:0-LPE, 17:1-LPI, 17:0-LPS, 17:0-LPG and 17:0-LPA. Most of the lipids, including LPC and LPE were extracted in the organic layer. The first lipid extract was dried under a stream of nitrogen gas, reconstituted with 0.5 ml of methanol/water mixture (1:1, v/v) containing 5 mM ammonium formate. The remaining aqueous layer was acidified to pH 2–2.5 with 1 N hydrochloric acid, and acidic polar lipids such as LPA, LPS, LPG, LPI and S1P were extracted to the organic layer by the method of Bligh and Dyer. The second lipid extract was dried under a stream of nitrogen gas and dissolved in 0.1 ml of a mixture of methanol/water (95:1, v/v) containing 5 mM formic acid for LC–MS/MS.

2.6. LC–MS/MS of LPL

LC–MS/MS was performed on a quadrupole-linear iontrap hybrid MS, 4000 QTRAP™ (Applied Biosystems/MDS Sciex; Concord, ON, Canada), with an Agilent 1100 LC system combined with an autosampler (HTS PAL, CTC Analytics, Zwingen, Switzerland), as previously described (Tokumura et al., 2009). Separation of LPCs and LPEs in the first lipid extract by LC was achieved using an Agilent ZORBAX Eclipse XDB-C18 column (50 mm × 1 mm; 3.5-μm particle size silica). The composition of the mobile phase was methanol/water (4:1, v/v) containing 5 mM ammonium formate, which was pumped at a flow rate of 0.1 ml/min for isocratic elution. Separation of LPAs, LPIs, LPGs, LPSs and S1Ps in the second lipid extract by LC was performed with a Tosoh TSK-ODS-100Z column (150 mm × 2 mm; 5-μm particle size silica) developed with methanol/water (19:1, v/v) containing 5 mM ammonium formate at a flow rate of 0.22 ml/min in an isocratic elution mode. At regular intervals, 5 μl aliquots of test solutions were applied to the mass spectrometer for analysis. LPL were analyzed by multiple reaction monitoring (MRM) in positive ion mode for LPC and LPE or in negative ion mode for LPA, LPS, LPG, LPI and S1P. In the positive ion MRM, Q1 and Q3 were set for the protonated molecular ion and $[phosphorylcholine]^+$ at *m/z* 184 for LPC and $[M+H - phosphoethanolamine]^+$ for LPE. In the negative ion MRM, Q3 was set to $[cyclic\ glycerol\ phosphate]^-$ at *m/z* 153 for LPA, LPG and LPS, $[inositolphosphate - H_2O]^-$ at *m/z* 241 for LPI and $[dehydrated\ phosphate]^-$ *m/z* 79 for S1P in combination with the deprotonated molecular ion as Q1. The amounts of the different molecular species of LPC and LPE were calculated from the ratios of their areas of positive ions to those of internal standards 17:0-LPC (25 ng/ml) and 17:0-LPE (12.5 ng/ml), respectively. Similarly, the amounts of molecular species of LPA, LPI and S1P were calculated from the ratios of their peak areas of negative ions to those of internal standards 17:0-LPA (1.25 ng/ml), 17:1-LPI (0.5 ng/ml), 17:0-LPS (1.25 ng/ml), 17:0-LPG (1.25 ng/ml) and 17:0-S1P (1.25 ng/ml), respectively.

2.7. Assay of choline-producing activity due to lysoPLD

The choline-producing activity of 10 fold-diluted plasma was measured by enzyme-coupled fluorometric determination of choline released from 0.15 mM 16:0-LPC at 37 °C for 24 h as previously reported (Tokumura, 2002). We expressed the activity in the aqueous fluid as nmol of choline produced in 24 h/ml of the undiluted plasma.

2.8. Assay of ecto-lipid phosphate phosphatase (LPP) activity

The LPP activity of 12 fold-diluted blood with saline was measured by determining phosphorus released from 2 mM 18:1-LPA at 37 °C for 60 min, as previously reported (Ino et al., 2012). The activity is expressed as nmol of phosphorus produced per ml of the undiluted blood during 24 h.