Short Communication

Cadmium-Induced Apoptosis in the Proximal Tubules of Rat Kidney

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DNA fragmentation is a biochemical marker of apoptosis, which is a controlled form of cell deletion induced by a series of physiological, chemical and biological stimuli. We previously reported that exposure of cultured renal cells to cadmium (Cd) resulted in an induction of DNA fragmentation¹⁾. Such a phenomenon is interesting because Cd-induced toxicity has been linked to renal necrosis²⁾, which is a cell death distinct from apoptosis. It has been reported that Cd caused damage predominantly in the proximal tubules²⁾. Although there were a few reports about apoptosis caused by Cd³⁻⁵⁾, no attempts have been made to evaluate whether or not apoptosis is cell-selectively induced by Cd while the metal-promoted necrotic damage occurs. This communication describes evidence for apoptosis of rat renal proximal tubules caused by Cd.

Materials and Methods

Animal treatments: Cd-MT-II was purified from livers of rats given CdCl₂ (2 mg Cd/kg/day × 5 days, s.c.) as previously described⁶⁾. Cd-MT-II was dissolved in saline and administered to male Wistar rats (8 weeks old, Clea Japan, Tokyo, Japan, 4-12 rats per group) by i.v. injection at a dose of 0.15 mg Cd/kg. Rats were killed under ether anesthesia by withdrawing an excess volume of blood from the heart at various times for collection of kidney and urine samples in time course studies. For control, rats without Cd-MT administration were used.

DNA fragmentation analysis: Cellular DNA in one kidney (1 g) from one rat per group was extracted in 5 mM Tris-HCl (pH 7.4)-20 mM EDTA-0.5% Triton X-100, as described previously¹⁾. Each sample was treated with RNase A and electrophoresed on a 1.2% agarose gel containing 0.5 μg/ml of ethidium bromide.

Measurement of Cd concentration in kidney and blood: Samples from the other kidney and blood (200 mg) were digested in concentrated nitric acid

at 110°C for 24 hr and the Cd levels were measured by inductively-coupled plasma-atomic emission spectrometry (ICP-AES) as described previously⁷⁾.

Evaluation of nephrotoxicity: For determination of Cd-caused nephrotoxicity⁸⁾, alkaline phosphatase (ALP) activity in urine was measured with a commercial kit from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Urinary creatinine was determined with a creatinine assay kit (Wako Pure Chemical Industries Ltd.). Urinary ALP activity was expressed as units per mg of creatinine.

Identification of apoptotic cells in the kidney: Apoptotic cells in the kidney were identified by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labelling (TUNEL) method⁹⁾. After TUNEL staining, specimens were counterstained with Carrazzi's hematoxylin.

Results and Discussion

To produce a specific acute kidney injury, we chose a single intravenous injection of Cd-MT (0.15 mg Cd/kg) into rats^{8, 10)}. The metal levels reached a maximum ($20.4 \pm 3.0 \mu\text{g/g}$) at about 3 hr after the injection and then declined gradually but the steady-state level, which is about the half of Cd concentration at 3 hr, was maintained up to 48 hr ($9.5 \pm 1.6 \mu\text{g/g}$). In contrast, the blood concentrations during the experiment were extremely low ($<0.36 \mu\text{g/g}$). A significant increase in ALP activity in urine was detected 48 hr after the Cd-MT administration: At the same time, marked morphological changes (i.e., damage to the brush border and cell loss in the proximal tubules of the kidneys) were also confirmed by light microscopy (see Fig. 2B).

Figure 1 shows a result of agarose gel electrophoresis of low molecular weight DNA extracted from the kidney tissues to detect oligonucleosomal DNA fragmentation. DNA fragmentation was detectable as early as 12 hr when renal injury as assessed by the detection of urinary ALP activity was negligible. Thus these data indicate that apoptosis evidenced by DNA fragmentation seemed to precede damage to cell membranes. The DNA ladders caused by internucleosomal DNA cleavage were

Fig. 1. Formation of renal DNA ladders after the injection of Cd-MT to rats. Lane 1, size markers given in base pairs; lane 2, control; lane 3, 3 hr; lane 4, 7 hr; lane 5, 12 hr; lane 6, 16 hr; lane 7, 24 hr; lane 8, 48 hr.

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Fig. 2. *In situ* detection of apoptotic cells (A) and morphological changes (B) in kidney sections of Cd-MT-treated rats. A, TUNEL staining renal specimen obtained at 24 hr after administration; B, PAS staining section at 48 hr after administration. Arrows indicate apoptotic cells. G, glomerulus; D, distal tubule; *, damaged proximal tubule.

detected for at least 48 hr.

To identify what type of renal cell is involved in the DNA fragmentation, TUNEL staining, which is based on the labelling of 3'-OH DNA ends generated by DNA fragmentation through the process of apoptosis¹¹⁾, was employed for the kidney sections (Fig. 2). In the specimen at 24 hr after Cd-MT administration, the proximal tubules, where necrotic damages predominantly take place at 48 hr after the administration (Fig. 2B), did contain apoptotic cells but the distal tubules or glomeruli did not (Fig. 2A). In contrast, almost no apoptotic cells were found in the kidneys of the control rat. The renal section at 24 hr after Cd-MT administration in the absence of exogenous terminal deoxynucleotidyl transferase also had no positive staining, indicating the specific color reaction in the experimental condition (data not shown).

In summary, the present observations demonstrate that previous *in vitro* findings, that Cd causes an induction of DNA fragmentation in cultured kidney cells¹⁾, translate to an *in vivo* situation of renal apoptosis. The apoptotic phenomenon caused in the proximal tubules was initiated before a significant leakage of ALP in urine occurred in our model. Since Cd-caused nephropathy has been recognized as a lesion in the proximal tubules²⁾, the present results indicate, therefore, that Cd-induced renal apoptosis may be an early and possibly causal event during experimental kidney failure triggered by Cd.

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References

1) Ishido M, Homma ST, Leung PS, Tohyama C.

Cadmium-induced DNA fragmentation is inhibitable by zinc in porcine kidney LLC-PK₁ cells. *Life Sci* 1995; 56: PL351-PL356.

- 2) Murakami M, Cain K, Webb M. Cadmium-Metallothionein-induced nephropathy: a morphological and autoradiographic study of cadmium distribution, the development of tubular damage and subsequent cell regeneration. *J Appl Toxicol* 1983; 3: 237-244.
- 3) El Azzouzi B, Tsangaris GT, Pellegrini O, Manuel Y, Benevise J, Thomas Y. Cadmium induces apoptosis in a human T cell line. *Toxicology* 1994; 88: 127-139.
- 4) Xu C, Johnson JE, Singh PK, Jones MM, Yan H, Carter CE. In vivo studies of cadmium-induced apoptosis in testicular tissue of the rat and its modulation by chelating agent. *Toxicology* 1996; 107: 1-8.
- 5) Tanimoto A, Hamada T, Koide O. Cell death and regeneration of renal proximal tubular cells in rats with subchronic cadmium intoxication. *Toxicol Pathol* 1993; 21: 341-352.
- 6) Tohyama C, Shaikh ZA. Metallothionein in plasma and urine of cadmium-exposed rats determined by a single-antibody radioimmunoassay. *Fund Appl Toxicol* 1981; 1: 1-7.
- 7) Homma S, Sasaki A, Nakai I, Sagai M, Koiso K, Shimojo N. Distribution of copper, selenium, and zinc in human kidney tumors by nondestructive synchrotron radiation X-ray fluorescence imaging. *J Trace Elem Exp Med* 1993; 6: 163-170.
- 8) Sugihira N, Sagai M, Suzuki KT. Renal damage induced by cadmium-metallothionein: Effects on biochemical indicators. *Toxicology* 1987; 44: 1-11.
- 9) Gavrieli Y, Sherman Y, Bern-Sasson SA. Identification of programmed cell death in situ via specific labelling of nuclear DNA fragmentation. *J Cell Biol* 1992; 119: 493-501.
- 10) Min KS, Kobayashi K, Onosaka A, Ohta N, Okada Y, Takana K. Tissue distribution of cadmium and nephropathy after administration of cadmium in several chemical forms. *Toxicol Appl Pharmacol* 1986; 86: 262-270.
- 11) Surh CD, Sprent J. T-cell apoptosis detected in situ during positive and negative selection in the thymus. *Nature* 1994; 372: 100-103.