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Short Communication

Histopathological localization of cadmium in rat placenta by LA-ICP-MS analysis

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Abstract: In order to clarify the histological localization of cadmium (Cd) in the placenta, we analyzed paraffin sections of placentas from rats with a single Cd exposure on gestation day 18 by the LA-ICP-MS imaging method compared with the histopathological changes. The placentas were sampled at 1 hour, 2 hours, 3 hours, 6 hours, and 24 hours after treatment. Histopathologically, the trophoblasts in the labyrinth zone of the Cd group showed swelling at 1 hour. At 2 and 3 hours, the trophoblasts showed swelling and vacuolar degeneration. At 6 and 24 hours, the syncytiotrophoblasts selectively underwent necrosis/apoptosis, resulting in a decrease in number. Remarkable metallothionein expression was observed in the trophoblastic septa, particularly cytotrophoblasts at 24 hours. The LA-ICP-MS analysis detected the localization of Cd in the fetal part of the placenta from 1 hour onwards. In particular, the intensity of Cd was prominent in the labyrinth zone and tended to increase with the progression of trophoblastic septa damages. The LA-ICP-MS analysis using the paraffin sections detected the localization of Cd in the fetal part of the placenta, and this methodology will be one of the valuable tools to detect heavy metals in toxicological pathology. (DOI: 10.1293/tox.2016-0022; J Toxicol Pathol 2016; 29: 279–283)

Key words: cadmium, LA-ICP-MS, placenta, rat

Cadmium (Cd) is known to be one of the most toxic heavy metals that induce damage to various organs, which is caused by a diversity of toxic effects[1]. Cd induces nephrotoxicity, osteotoxicity, lung toxicity, hepatotoxicity, reproductive toxicity, carcinogenicity, and teratogenicity. Regarding reproductive toxicity, Cd exposure during pregnancy in mice, rats, and hamsters results in teratological effects, such as skeletal malformations and exencephaly in the early gestation stage and fetal death and placental necrosis in the late gestation stage. Although Cd can cross the placenta and accumulates in fetal tissues, fetal toxicity in rats is considered to be caused by Cd-induced placental or maternal dysfunction, not by a direct effect of Cd on fetuses[2]. The placenta is known to be a primary target for Cd toxicity during pregnancy[3] and is one of the major Cd accumulation tissues in rats[4]. Histologically, the rat placenta is composed of the fetal part (labyrinth zone and basal zone) and the maternal part (decidua and metrial gland)[5, 6]. However, the histological localization of Cd in each part of the placenta has not been reported.

In recent years, elemental detection of thin tissue sections by means of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has been developed for imaging trace elements (metals, metalloids, and nonmetals) and isotopes in biological materials, providing accurate and reliable data for quite different applications[7]. LA-ICP-MS analysis has become the method of investigation of elemental distributions in biological tissue sections due to its high sample throughput, high sensitivity, and spatial resolution down to 4 μm. Cryosections[7, 8] or fixed sections[9, 10] are possible sources for elemental bioimaging with LA-ICP-MS. Therefore, LA-ICP-MS analysis is becoming one of the important tools for pathology[11]. In the present study, in order to clarify the histological localization of Cd in the placenta, we used the LA-ICP-MS imaging method to analyze paraffin sections of placentas from rats exposed to Cd.

Non-pregnant specific pathogen-free Wistar Hannover rats (Japan Laboratory Animals, Inc., Tokyo, Japan) were purchased at approximately 10–14 weeks of age. Each female rat was housed together with a male rat. The occurrence of copulation was established by daily inspection for a vaginal plug. Mated female rats were utilized in this study. Gestation day (GD) 0 was designated as the day on when the presence of a vaginal plug was identified. The animals were single-housed in plastic cages on softwood chip bed-

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ding in an air-conditioned room (22 ± 2°C; humidity, 55 ± 10%; light cycle, 12 hr/day). Feed (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water were available *ad libitum*. Fifteen pregnant rats were randomly allocated to the control group of 5 rats and the Cd group of 10 rats. Cadmium chloride ($CdCl_2$) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in sterile 0.75% saline solution. $CdCl_2$ was subcutaneously administered to rats at doses of 0 mmol/kg with sterile 0.75% saline solution (the control group) or 0.04 mmol/kg $CdCl_2$ (the Cd group) with a volume of 1 ml/100 g body weight on GD 18. Previously, treatment with this dose level on GD 18 was reported to induce fetal death and placental necrosis[12]. All treatments were performed between 7 and 9 a.m. One dam in the control group and two dams in the Cd group each were sampled at 1 hour, 2 hours, 3 hours, 6 hours, and 24 hours after treatment. The dams were euthanized by exsanguination under anesthesia and necropsied. All fetuses were removed from the placentas. Five placentas/litter were randomly obtained from embryos/fetuses of all dams in both groups at the sampling time points. All placentas were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm thickness, and stained routinely with hematoxylin and eosin (HE) for histopathological examination. In order to figure the localization of Cd, immunohistochemical staining of metallothionein (MT) antibody against rat MT-1/MT-2 (MTE9, DakoCytomation, Carpinteria, USA) was performed according to the avidin-biotin complex (ABC) method (VECTSTAIN ABC Kit, Vector Laboratories, Inc., Burlingame, California, USA). These experiments were conducted according to the Guidelines for Animal Experimentation, Japanese Association for Laboratory Animal Science.

An LSX 213 laser ablation system (Teledyne CETAC Technologies, Omaha, USA) working at a wavelength of 213 nm with a scanning camera was coupled to a quadruple-based iCAP Qc ICP-MS (Thermo Fisher Scientific, Waltham, USA) in such a way that the aerosol from the laser ablation unit was directly introduced into the injector pipe of the ICP-MS by a carrier gas through a tygon tube (Fig. 1). The laser parameters, such as laser energy, scan rate and frequency, were optimized to receive accurate lateral elemental information. A spot size of 150 um was selected. The scan rate was 50 um/s. The used laser energy was adjusted to 15.13 mJ, while the laser was operated at a shot repetition rate of 20 Hz. For the present study, a HelEx™ 2-volume cell was utilized. For tuning of the instrument, a fully automated adjustment approach was programmed using the provided functionality of the Qtegra software. For this purpose, parameters like the position of the torch, extraction voltage, and additional carrier gas flow of argon, as well as the most relevant ion lenses in front of the mass analyzer, were optimized for maximum intensity and low levels of oxides and doubly charged ions. As a carrier gas (0.65 L/min), helium was used to obtain an improved washout behavior. Additional ICP-MS conditions were as follows: a nickel sampler and skimmer without insert and a quartz injector pipe with an inner diameter of 3.5 mm; RF power, 1,550 W; auxiliary

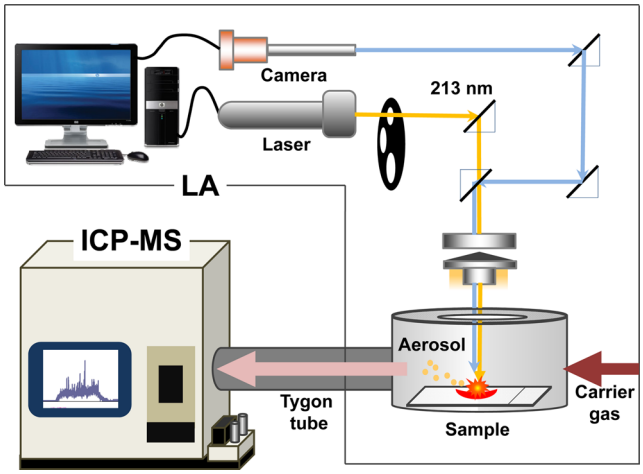

Fig. 1. LA-ICP-MS. Laser ablation system (LA): sample extraction from an unstained paraffin section slide by laser ablation with a 213 nm laser beam with camera scanning. Inductively coupled plasma-mass spectrometry (ICP-MS): analysis of a sample-derived aerosol from LA.

gas flow rate, 0.8 L/min; dwell time, 0.22 s; and isotopes monitored, ^{111}Cd and ^{114}Cd. Because of the absence of isobaric interference for the investigated mass to charge ratios (blank signal, 400 cps), the analysis was performed in the standard mode of the ICP-MS instrument. One placenta on the unstained paraffin section slide from one dam at each sampling time in the both groups was measured with the LA-ICP-MS under the above condition. Placentas showing typical lesions at each sampling time were selected for the LA-ICP-MS analysis. After the LA-ICP-MS analysis, the used slides were stained routinely with HE staining for histopathological examination.

There were no deaths of dams in the either group. Fetal death was observed from 6 hours onwards after treatment in the Cd group (Fig. 2). Histopathologically, the trophoblasts in the labyrinth zone of the Cd group showed swelling at 1 hour (Fig. 2). At 2 and 3 hours, the trophoblasts showed swelling and vacuolar degeneration (Fig. 2). At 6 and 24 hours, the syncytiotrophoblasts selectively underwent necrosis/apoptosis resulting in a decrease in number. Some placentas showed congestion and hemorrhage, resulting from thinning of the trophoblastic septa (Fig. 2). During the experimental period, there were no lesions in the basal zone, decidua, or metrial gland in the Cd group or in any zones in the control group.

The MT expression at each sampling time point in each zone is shown in Table 1. In the labyrinth zone, remarkable MT expression was observed in the trophoblastic septa, particularly in cytotrophoblasts at 24 hours in the Cd group, compared with in the control group (Fig. 2). There was no remarkable difference in MT expression in the basal zone, decidua, or metrial gland at any sampling time between the control and Cd groups.

The LA-ICP-MS intensity of Cd at each sampling time

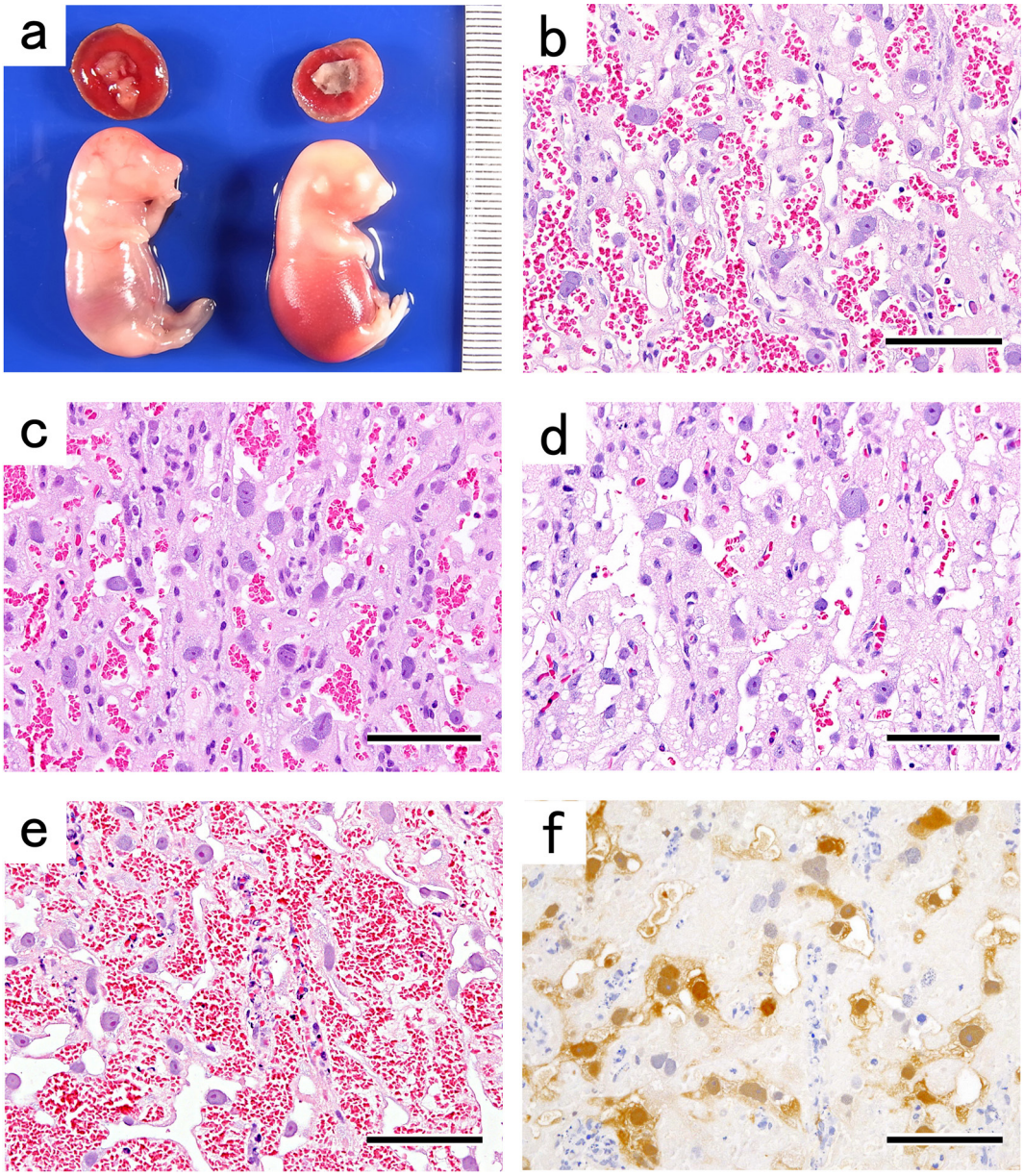

Fig. 2. Gross appearance and histopathological changes in labyrinth zone. a) Gross appearance of fetus and placenta at 24 hours after treatment. Control group (left) and Cd group (right). In Cd group, macerated fetus immediately after death and geographic discoloration of fetal surface of placenta. b) At 1 hour in control group. (HE staining) c) At 1 hour in Cd group. Swelling of trophoblasts. (HE staining) d) At 3 hours in Cd group. Swelling and vacuolar degeneration of trophoblasts. (HE staining) e) At 24 hours in Cd group. Selective necrosis/apoptosis of syncytiotrophoblasts. Congestion and hemorrhage with thinning of trophoblastic septa. (HE staining) f) At 24 hours in Cd group. MT expression in trophoblastic septa, particularly cytotrophoblasts. (MT immunohistochemical staining) Bar = 100 μm.

point in each zone is shown in Table 1 and Figure 3. In the labyrinth zone, the intensity of Cd was detected from 1 hour onwards and showed a marked increase at 24 hours in the Cd group. In the basal zone, the intensity of Cd was detected at 1 hour, 2 hours, and 24 hours in the Cd group, but the levels were less than those in the labyrinth zone. The intensity of Cd could not be detected in the decidua or metrial gland in the Cd group, or in any zones in the control group.

These results revealed that the LA-ICP-MS analysis using the paraffin sections detected the localization of Cd in the fetal part of the placenta, but not in the maternal part of the placenta. In particular, the intensity of Cd was prominent in the labyrinth zone and tended to increase with the progression of trophoblastic septa damage. Immunohistochemically, MT expression was increased in the labyrinth zone, although it was only detected at only 24 hours. It has been known that MT expression is observed in the yolk sac and decidua, but not in the labyrinth zone, in the normal

Table 1. LA-ICP-MS Intensity and MT Immunohistochemical Expression in Placenta

Part of placenta	LA-ICP-MS intensity (average of cps in part)						MT immunohistochemical expression					
	Control	Cadmium					Control	Cadmium				
		1 hr	2 hr	3 hr	6 hr	24 hr		1 hr	2 hr	3 hr	6 hr	24 hr
Labyrinth zone	N.D.	3,690	4,305	5,419	3,714	7,099	−	−	−	−	−	+++
Basal zone	N.D.	616	439	N.D.	N.D.	821	±	±	±	±	±	±
Decidua basalis	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	±	±	±	±	±	+
Metrial gland	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	±	±	±	±	±	±

N.D.: not detected. −, negative; ± , minimal, +, mild; ++, moderate; +++, severe.

developing rat placenta[13]. Therefore, the MT immunohistochemical data seems to support the results of the localization of Cd by the LA-ICP-MS analysis broadly. In addition, it is considered that the positive reaction of MT immunohistochemistry was observed 6 hours or more than after Cd deposition at earliest. LA-ICP-MS analysis was sensitive tool to detect Cd deposition in the paraffin sections compared with MT immunohistochemistry.

The fetal part of the placenta on GD 19 is primarily composed of four kinds of trophoblasts; cytotrophoblasts and syncytiotrophoblasts in the labyrinth zone and spongiotrophoblasts and trophoblastic giant cells in the basal zone[14]. It has been known that Cd mainly injuries the syncytiotrophoblasts as a result of the cellular and mitochondrial damage and that the cytotrophoblasts remain relatively unaffected early stage in the labyrinth zone[12, 15]. In the present study, although Cd deposition was observed in these trophoblasts, the highest affinity cells for Cd were cytotrophoblasts, and the most severely damaged cells were spongiotrophoblasts. Thus, it is considered that the sensitivity to Cd toxicity is different among these trophoblasts. Further detailed investigations of the treatment on an earlier gestation day are necessary to clarify the differential sensitivity of the Cd toxicity among these trophoblasts.

It is known that LA-ICP-MS can be applied as microscopic detector of exogeneous heavy metals (Cu, Fe, Zn, Pt, etc.) at the tissue level[9, 16]. In addition, paraffin section slides that have been analyzed with LA-ICP-MS can be subjected to HE staining or special staining. Furthermore, the HE-stained slides can also be subjected to LA-ICP-MS analysis[16]. Therefore, it is possible to identify the location of metal deposition by LA-ICP-MS analysis in comparison with histopathological changes. The LA-ICP-MS methodology will be exploited in future toxicological pathology for a mode of action approach.

In conclusion, LA-ICP-MS analysis using paraffin sections detected the localization of Cd in the fetal part of the placenta, particularly in the labyrinth zone, and this methodology will be one of the valuable tools to detect heavy metals in toxicological pathology.

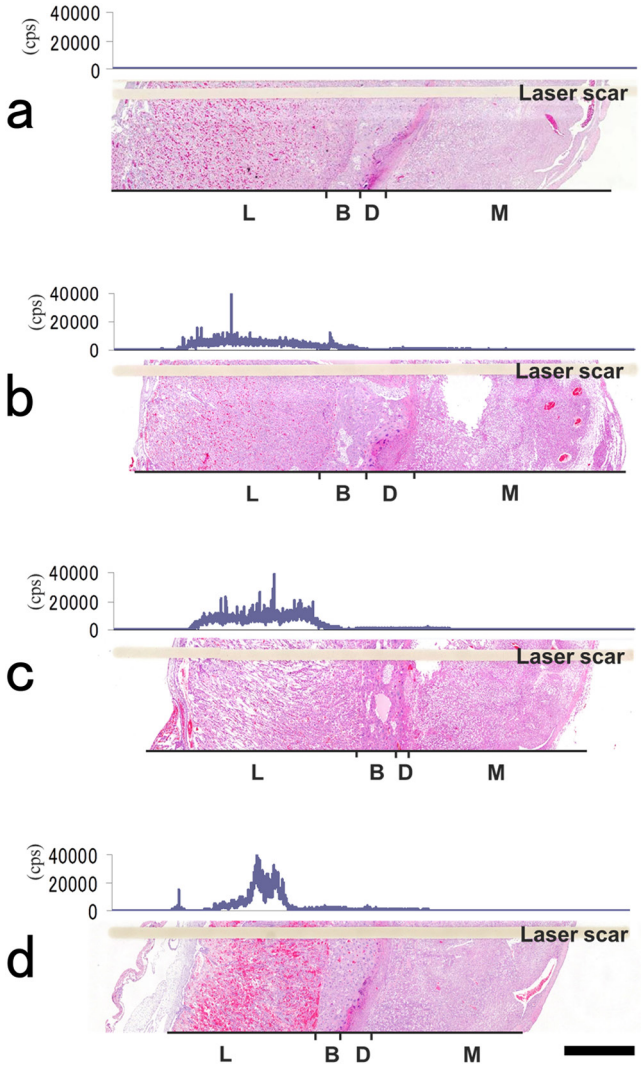

Fig. 3. LA-ICP-MS chromatography and histopathology. a) At 1 hour in control group. b) At 1 hour in Cd group. c) At 3 hour in Cd group. d) At 24 hour in control group. L, labyrinth zone; B, basal zone; D, decidua basalis; M, metrial gland. (HE staining) Bar = 1 mm.

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