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 effects1. 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 fetuses2. The placenta is known to be a primary target for Cd toxicity during pregnancy3 and is one of the major Cd accumulation tissues in rats4. 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 applications7. 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. Cryosections7, 8 or fixed sections9, 10 are possible sources for elemental bioimaging with LA-ICP-MS. Therefore, LA-ICP-MS analysis is becoming one of the important tools for pathology11. 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-
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 chlo-
ride (CdCl2) (Wako Pure Chemical Industries, Ltd., Osaka,
Japan) was dissolved in sterile 0.75% saline solution. CdCl2
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 CdCl2 (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 necrosis12. All treatments were performed be-
tween 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 embry-
os/fetuses of all dams in both groups at the sampling time
points. All placentas were fixed in 10% neutral buffered for-
malin, embedded in paraffin, sectioned at 4 μm thickness,
and stained routinely with hematoxylin and eosin (HE) for
histopathological examination. In order to figure the local-
ization of Cd, immunohistochemical staining of metallo-
thionein (MT) antibody against rat MT-1/MT-2 (MTE9, Da-
koCytomation, Carpinteria, USA) was performed according
to the avidin-biotin complex (ABC) method (VECTSTAIN
ABC Kit, Vector Laboratories, Inc., Burlingame, Califor-
nia, USA). These experiments were conducted according to
the Guidelines for Animal Experimentation, Japanese As-
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 quadru-
ple-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 ele-
mental information. A spot size of 150 μm was selected. The
scan rate was 50 μm/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 HelExTM 2-volume
cell was utilized. For tuning of the instrument, a fully auto-
mated adjustment approach was programmed using the pro-
vided 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 opti-
mized 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. Addi-
tional 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
gas flow rate, 0.8 L/min; dwell time, 0.22 s; and isotopes moni-
tored, 111Cd and 114Cd. Because of the absence of iso-
baric 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 his-
opathological examination.

There were no deaths of dams in the either group. Fetal
defath 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 ne-
crosis/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, par-
ticularly 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
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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

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.
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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. 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. 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. 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. 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.

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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 1 hr 2 hr 3 hr 6 hr 24 hr | Cadmium 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. | ± ± ± ± ± ± |
| Metrial gland          | N.D. N.D. N.D. N.D. N.D. | ± ± ± ± ± ± |

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