

Short Communication

## Changes of MAO-A and MAO-B Expressions in the Placenta of MPTP or MPP<sup>+</sup> Treated Mice

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**Abstract:** In the present study, we evaluated the influence of intraperitoneal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) on the placenta. There was no increase in apoptotic cells in the placentas of C57BL/6 mice treated with 25.0 mg/kg MPTP or 17.1 mg/kg MPP<sup>+</sup>, indicating that a single injection of the chemicals may induce very slight cytotoxicity in the placenta at 12 hr after administration. The decrease in the expression of monoamine oxidase (MAO)-A in the labyrinth zone and that of MAO-B in the basal zone may be due to the decrease in cell activity, whereas the increase of MAO-B expression in the labyrinth zone after MPTP treatment may be due to a responsive reaction caused by MPTP, one of the substrates of MAO-B. The results represent histological evidence that MAO-B may be involved in the metabolism of MPTP to MPP<sup>+</sup> in the labyrinth zone of the mouse placenta. In the present study, no increase in apoptotic cells indicates that MPTP and MPP<sup>+</sup> are hardly toxic to the placenta, and the histological change in MAO-B expression may indicate the possibility of involvement of placental MAO-B in MPTP metabolism. (DOI: 10.1293/tox.26.73; J Toxicol Pathol 2013; 26: 73–77)

**Key words:** C57BL/6 mice, placenta, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), MPP<sup>+</sup> (1-methyl-4-phenylpyridinium), MAO-B (monoamine oxidase B)

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity has been demonstrated to cause damage to the central nervous system (CNS), especially to the dopaminergic neurons in the nigrostriatal system<sup>1–4</sup>. In adult C57BL/6 mice, MPTP enters into the brain through the blood-brain barrier (BBB) and is converted to 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) by monoamine oxidase (MAO)-B<sup>5,6</sup>. MPP<sup>+</sup> is then taken up into a neuronal cell and ultimately causes cell death<sup>7,8</sup>.

There has been much research on the neurotoxicity of MPTP in the mouse embryo. Chronic MPTP exposure during the organogenesis period reduced the fetal weight, motor activity, number of striatal tyrosine hydroxylase (TH)-positive cells, and amounts of dopamine and homovanillic acid<sup>9</sup>. In our previous study, a single MPTP or MPP<sup>+</sup> exposure at pregnancy day (PD) 12 led to an increase in apoptotic cells and decrease in TH-positive cells in the CNS of the embryo<sup>10</sup>. MPTP and MPP<sup>+</sup> injected into a dam and reaching the embryo would have to have crossed the placenta.

However, there are only a few reports dealing with the placental transmission of MPTP<sup>11,12</sup>.

In the embryonic part of the placenta, maternal and embryonic blood is separated by the trophoblast layer in the labyrinth zone<sup>13,14</sup>. A variety of materials such as oxygen, nutrients, waste products, and so on are exchanged between the maternal and embryonic plasma through this layer<sup>13</sup>. The trophoblast layer is composed of syncytiotrophoblasts, cytotrophoblasts, connective tissue and the embryonic capillary endothelium<sup>15,16</sup>, and the penetration limit of materials through this layer is called the “blood-placental barrier”<sup>14,15</sup>. On PD 12 in the mouse, maternal-embryonic transfer occurs primarily through the definitive chorioallantoic placenta, although mice also possess an inverted yolk sac placenta that can function as a major conduit for transfer<sup>16</sup>. Low molecular weight materials, large molecules that undergo pinocytosis or receptor-mediated endocytosis (like IgG) can gain access to the embryonic blood stream<sup>16</sup>.

In the present study, we evaluated the influence of MPTP on the placenta and metabolism from MPTP to MPP<sup>+</sup> in the placenta.

Pregnant C57BL/6J mice at PD 11 were obtained from Charles River Laboratories Japan (Yokohama, Japan). They were housed in metal cages under controlled housing conditions (temperature, 23 ± 2°C; relative humidity, 55 ± 5%; light/dark cycle, 12/12 hr) and fed commercial pellets (MF; Oriental Yeast., Tokyo, Japan) and water *ad libitum*. MPTP-

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HCl (Sigma, St. Louis, MO, USA) and MPP-Iodide (Sigma) were dissolved in saline. In previous acute toxicity research, a single injection (about 30 mg/kg)<sup>17, 18</sup> of MPTP-HCl or a single (12.5–25.0 mg/kg) of MPP-Iodide<sup>19, 20</sup> was used. In a preliminary study, pregnant mice at PD 12 were injected i.p. with 12.5, 25.0 or 50.0 mg/kg MPTP-HCl (2 mice, respectively) or 8.6, 17.1 or 34.2 mg/kg MPP-Iodide (2 mice, respectively). The majority of the mice treated with 50.0 mg/kg MPTP-HCl or 34.2 mg/kg MPP-Iodide at PD 12 died (1 of 2 mice and 2 of 2 mice, respectively), and no changes in performance status were observed in the mice treated with 12.5 mg/kg MPTP-HCl or 8.6 mg/kg MPP-Iodide at PD 12 (1 of 2 mice and 2 of 2 mice, respectively). Hence injection doses of 25.0 and 17.1 mg/kg were determined for MPTP-HCl and MPP-Iodide, respectively. Eight pregnant mice were injected i.p. with 25.0 mg/kg MPTP-HCl (4 mice) or 17.1 mg/kg MPP-Iodide (4 mice) at PD 12. As a control, 4 mice were i.p. injected with an equivalent volume of saline (10.0 ml/kg). At 12 hr after treatment, mice were sacrificed by exsanguination under a deep anesthesia with diethyl ether. All procedures used in the present study were approved by the Committee of Animal Experiments of the Graduate School of Agricultural and Life Sciences, the University of Tokyo.

Two randomly chosen placentas per dam were fixed in a 10% neutral-buffered formalin solution. Each placenta was cut along the central axis into 2 pieces, and one of the pieces was embedded in paraffin and a sliced serially from the cut surface to obtain large sections and observe both central and peripheral zones. Four  $\mu\text{m}$ -thick sections were stained with hematoxylin-eosin (HE). Apoptotic cells containing fragmented DNA were detected by the TdT-mediated dUTP nick end labeling (TUNEL) method using an ApopTag Peroxidase *In Situ* Apoptosis Detection Kit (Millipore, Temecula, CA, USA) according to the manufacturer's instructions. The histological sections were then counterstained with methyl green. Immunohistochemistry was performed by the EnVision method (Dako, Carpinteria, CA). Deparaffinized sections were first autoclaved at 120°C for 15 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval. The sections were then treated with 1% hydrogen peroxide-methanol at room temperature for 30 min and then with 8% skim milk-Tris-buffered saline at 37°C for 30 min for protection from nonspecific reactions. The sections were then incubated with one of the following primary antibodies, which have species specificity for the human, mouse and rat, at 4°C for 18 hr: rabbit anti-cleaved caspase-3 (1:200; Cell Signaling Technology, Danvers, MA, USA), rabbit anti-MAO-A (1:300; Proteintech Group, Chicago, IL, USA) and rabbit anti-MAO-B (1:30; Proteintech Group). Following the secondary antibody reaction with the EnVision Anti-Rabbit Conjugation System (Dako) at 37°C for 1 hr, the reaction products were finally visualized with 0.05% 3,3'-diaminobenzidine and 0.03% hydrogen peroxide in Tris-HCl buffer. Counterstaining was performed with methyl green.

MAO-A- and MAO-B-positive areas in the placenta were measured using image analysis software, ImageJ (NIH, Bethesda, MD, USA) and Adobe Photoshop 6.0 (Adobe Sys-

tems, San Jose, CA, USA). All results are expressed as the mean  $\pm$  standard error (SE). Statistical analyses were carried out with the *F*-test and Student's *t*-test or Aspen-Welch test to assess differences between data groups. Differences were considered significant when  $P < 0.05$ .

Decreases in locomotor activity, hypothermia and convulsion were observed in the majority of MPTP or MPP<sup>+</sup> treated pregnant mice. No significant morphological changes were observed in the placentas of the MPTP or MPP<sup>+</sup> treated mice (Fig. 1b–g). Very few apoptotic (TUNEL-positive and/or cleaved caspase-3-positive) cells were observed in the labyrinth zone of the placenta in the control and MPTP- or MPP<sup>+</sup>-treated mice (data not shown). MAO-A was expressed in the trophoblast and capillary endothelium of the labyrinth zone (Fig. 2a–c) of the mice, and the positive area was decreased by treatment with either MPTP or MPP<sup>+</sup> (Fig. 2d). However, the expression was not observed in the basal zone (data not shown). MAO-B was expressed in the trophoblast and capillary endothelium in the labyrinth zone (Fig. 3a–c) and spongiotrophoblast and trophoblastic giant cell in the basal zone (Fig. 3d–f) of mice. In the labyrinth zone, the MAO-B-positive area was increased by treatment with MPTP (Fig. 3g). However, in the basal zone, the area was decreased by treatment with either MPTP or MPP<sup>+</sup> (Fig. 3h).

There were no morphological changes and no increases in apoptotic cells in the placentas of mice treated with MPTP or MPP<sup>+</sup>, indicating that a single injection of MPTP or MPP<sup>+</sup> does not induce severe cytotoxicity (e.g., irreversible changes) in placental cells.

MAO-A-immunopositive cells were observed only in the labyrinth zone, not in the basal zone, which was in accordance with the results of a previous study<sup>21</sup>; however, MAO-B-positive cells were observed in both the labyrinth zone and basal zone as in previous studies<sup>22, 23</sup>. The results indicate that the antibodies used in the present study have high specificity for MAO-A and MAO-B, respectively.

Immunohistochemical expressions of MAO-A in the labyrinth zone and MAO-B in the basal zone were decreased by treatment with MPTP or MPP<sup>+</sup>. Such decreases in MAO-A and MAO-B may be related to the cytotoxicity in the spongiotrophoblast and trophoblast induced by MPTP and MPP<sup>+</sup>, though we have no other data from quantitative analyses, such as biochemical assays with internal controls. On the other hand, a significant increase in MAO-B-positive cells in the labyrinth zone 12 hr after MPTP treatment was demonstrated in the present study, in accordance with the results of a previous study on embryonic and newborn mice<sup>24</sup>. We speculate that the increase in MAO-B expression is a kind of responsive reaction to injected MPTP, a substrate for MAO-B. If so, MAO-B might be involved in the metabolism of MPTP to MPP<sup>+</sup> in the labyrinth zone of the mouse placenta.

Previous studies indicated that materials with molecular weights of less than 600 Da, including MPTP and MPP<sup>+</sup> (molecular weights are 173.3 Da and 170.3 Da, respectively), easily transfer to the embryo through passive diffusion<sup>25</sup>.

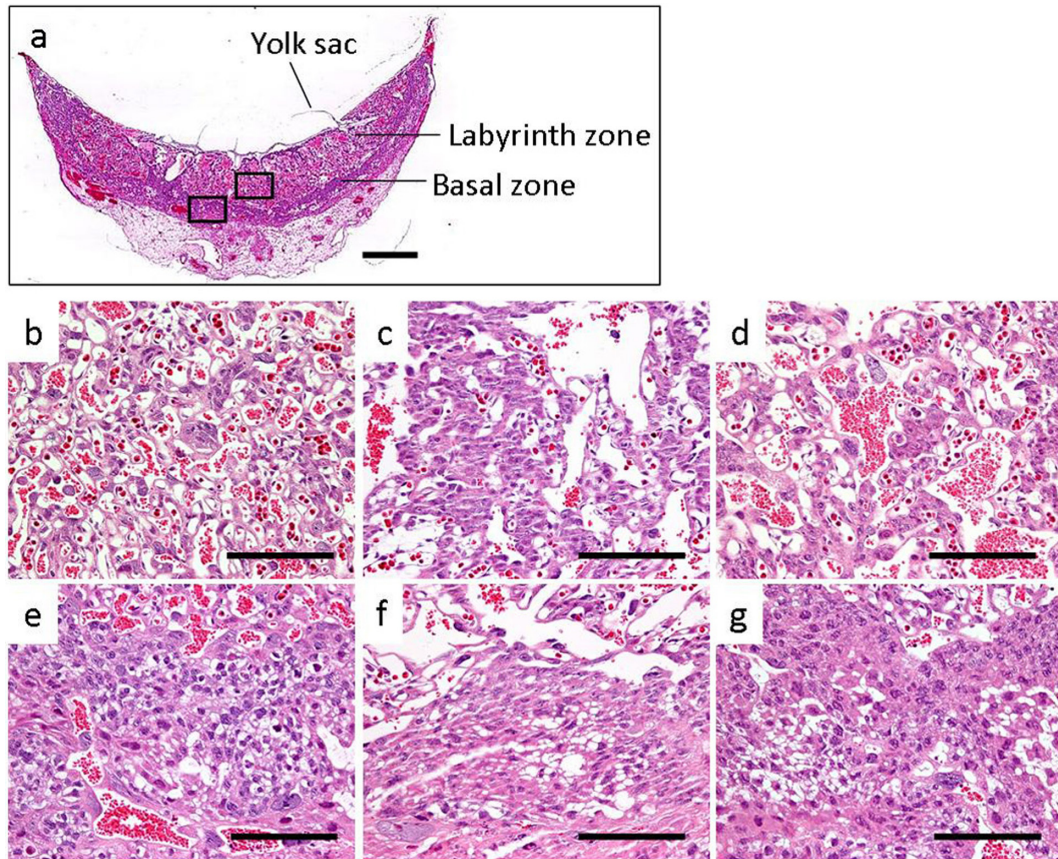


Fig. 1.

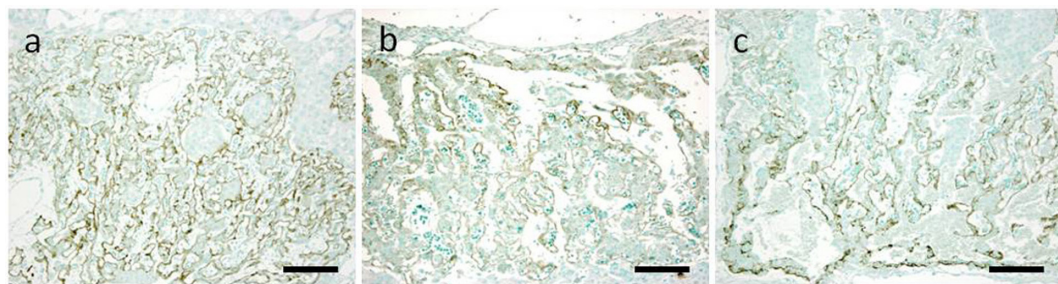
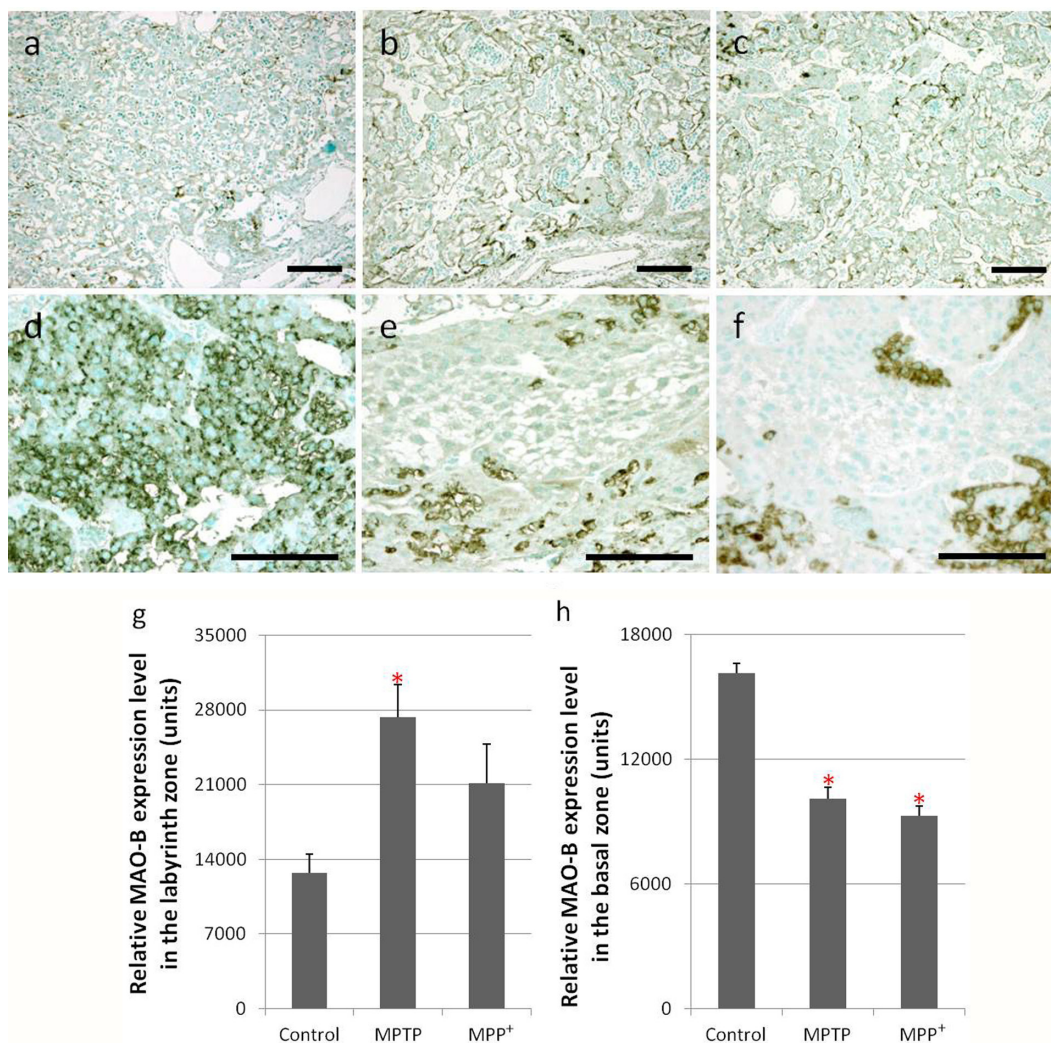


Fig. 2.

**Fig. 1.** A representative photographs of HE-stained sections of placenta (a). Both central and peripheral zones of the placenta were observed. The boxes indicate the sites shown in (b) to (g). No morphological changes were observed in the placentas of the mice treated with either MPTP or MPP<sup>+</sup>. Control (b and e), MPTP treated (c and f) and MPP<sup>+</sup> treated (d and g) mice. Labyrinth zone (b, c and d) and basal zone (e, f and g). Scale bars = 1 mm (a) and 100  $\mu$ m (b-g).

**Fig. 2.** Changes of MAO-A expression in the labyrinth zone of mice treated with MPTP or MPP<sup>+</sup>. MAO-A was expressed in the trophoblast and capillary endothelium of the labyrinth zone. Control (a), MPTP treated (b) and MPP<sup>+</sup> treated (c) mice. The MAO-A-positive area was measured. MAO-A expression was significantly decreased in the labyrinth zone of MPTP or MPP<sup>+</sup> treated mice (d). Scale bars = 100  $\mu$ m (a-c). Values are mean  $\pm$  SE (d). \* $P$ <0.05.



**Fig. 3.** Changes of MAO-B expression in the labyrinth zone and basal zone of mice treated with MPTP or MPP<sup>+</sup>. MAO-B was expressed in the trophoblast and capillary endothelium in the labyrinth zone and in the spongiotrophoblast and trophoblastic giant cell of the basal zone. Control (a and d), MPTP treated (b and e) and MPP<sup>+</sup> treated (c and f) mice. Labyrinth zone (a, b and c) and basal zone (d, e and f). MAO-B-positive areas in the labyrinth zone and basal zone were measured (g and h). MAO-B expression was significantly increased in the labyrinth zone of MPTP treated mice (g). MAO-B expression was significantly decreased in the basal zone of mice treated with MPTP or MPP<sup>+</sup> (h). Scale bars = 100 μm (a–f). Values are mean ± SE (g and h). \**P*<0.05.

We also showed the embryonic neuronal toxicity of MPTP or MPP<sup>+</sup> injected i.p. in the maternal body<sup>10</sup>. MPTP and MPP<sup>+</sup>, which is metabolized from MPTP, in the maternal body and placenta can pass through the placenta freely and transfer to the embryo.

The present study demonstrated that MPTP and MPP<sup>+</sup> are hardly toxic to the mouse placenta and that MAO-B expressed in the placenta may be involved in the metabolism of MPTP to MPP<sup>+</sup>. However, the mechanisms of the MAO-B expression change in the labyrinth zone and basal zone should be further examined.

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