論文の内容の要旨

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論文題目

Neurotoxicity of 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to neural progenitor cells in C57BL/6 mice

（C57BL/6 マウスの神経前駆細胞に対する 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine （MPTP）の毒性）

1-Methyl-4-Phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a byproduct of the chemical synthesis of a meperidine analog with potent heroin-like effects, which causes damage to dopaminergic neurons and depletes dopamine in a manner similar to that seen in Parkinson' disease (PD). When administered to animals, MPTP passes through the blood-brain barrier and is converted, mainly in glial cells, into its active form, 1-methyl-4-phenylpyridinium (MPP+), by an enzyme, monoamine oxidase B (MAO-B). For the last two decades, MPTP has been widely employed to generate animal models of PD in rodents, especially in C57BL/6 mice and non-human primates. In the present study, however, evidences have been provided that the exposure to MPTP stimulates a rapid cell death response in the subventricular zone (SVZ) and rostral migratory stream

(RMS) in the adult mouse brain, indicating that the neurotoxicity of MPTP is not restricted to nigrastriatal dopaminergic neurons. The SVZ in adult mice contains three distinct cell types: migrating neuroblasts (Type A cells), astrocytes (Type B cells), and novel putative precursor cells (transit amplifying cells, Type C cells). B cells as neural stem cells express GFAP and give rise to the rapidly dividing, transit-amplifying C cells, which generate A cells. A cells correspond to proliferating, migrating neuronal precursors and join an extensive tangential network of pathways for chain migration that feeds into the RMS leading to the OB. Findings of MPTP-induced cell death in the SVZ and RMS will lead to a better understanding of MPTP neurotoxicity and its application in PD animal models.

1. Demonstration of the classic neurotoxicity of MPTP: A feature of age-dependent susceptibility in C57BL/6 mice.

The present study was designed to evaluate dopaminergic neuronal loss in the substantia nigra pars compact (SNpc) with immunohistochemical staining. C57BL/6 mice were intraperitoneally injected four times with 15 mg/kg 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP), at 2 h intervals on 10 and 21 days, and 6, 12, 24 and 48 weeks of age. Animals were sacrificed 48 hours after the last injection. No change in the number of tyrosine hydroxylase (TH)-positive neurons was observed in 10- and 21-day-old mice after MPTP treatment compared with their corresponding controls. In contrast, MPTP produced a loss of 20.3% of TH-positive neurons in 6 week-old mice, and further decreases with advancing age, i.e., 35.8%, 39.9% and 56.2% TH-positive neuronal loss at 12, 24 and 48 weeks of age, respectively. These results provide evidence of age-related susceptibility of C57BL/6 mice to MPTP using TH immunohistochemistry. However, we failed to observe apoptosis of neurons in SNpc of mice of all ages after a subacute protocol of MPTP treatment (30 mg/kg/day × 5days).

2. MPTP induces apoptosis of neural progenitor cells in the SVZ and RMS in adult C57BL/6 mice.

In recent years, the notion that the neurotoxicity of MPTP is restricted to dopaminergic neurons in the substantia nigra has been challenged. Here, evidences have been provided that a single acute injection of MPTP (50 mg/kg) induced apoptosis in the SVZ and RMS in the adult C57BL/6 mouse brain. The number of TUNEL-positive cells peaked at 24 h after injection, and decreased thereafter, paralleling the change in the number of cleaved caspase-3-positive cells after MPTP injection. Results of immunohistochemistry and ultrastructural analyses indicated that the majority of apoptotic cells in the SVZ and RMS were migrating neuroblasts (Type A cells), while a few were astrocytes (Type B cells). No apoptosis occurred in transit-amplifying progenitors (Type C cells). The decrease in A cell numbers was most marked on day 2 and lasted to day 8 after the administration. A rapid and transient phagocytosis of apoptotic cells by microglial cells was demonstrated to parallel the MPTP-induced apoptosis.

The acute or subacute administration of MPTP has been widely used in C57BL/6 mice to develop models of Parkinson's disease. Here, dosage-dependent neurtoxicity of MPTP to neural progenitor cells was also investigated by using the acute or subacute paradigm.

3. Identification of genes involved in MPTP-induced neural progenitor cell apoptosis by Oligo Microarray.

The multipotential progenitor cells reside in SVZ and RMS are eliminated through apoptosis to maintain a balance for proper development of the mammalian nervous system. The mechanism of apoptotic death in the SVZ and RMS is attracting much interest and still remains to be clarified. To identify genes involved in the cell death induced by MPTP treatment, profiles of gene expression were analyzed using Oligo GEArray^{TM} Mouse Apoptosis Microarray with 112 genes and Oligo GEArray^{TM} Mouse DNA Damage Signaling Pathway Microarray with 113 genes. The total RNA was extracted from the olfactory bulbs isolated from saline- and MPTP-treated mice and amplified and labeled for hybridization to the Oligo GEArray with TrueLabeling-AMP™ 2.0. Hybridized arrays were scanned with a ChemiDoc XRS System. Data were acquired and

analyzed with GEArray Expression Analysis Suite. Three arrays were used for hybridization and only the genes with differential expression and at least a 2-fold discrepancy in all the three arrays were considered to associate with MPTP-induced neural progenitor cell (NPC) death.

In the analysis of genes involved in apoptosis pathway, it was found that 9, 15, and 10 genes were significantly increased (> 2-fold changes) in their expression when detected at 12, 24, and 36 hours, respectively; on the other hand, 15, 21, and 4 genes involved in DNA damage signaling pathway were also significantly increased (> 2-fold changes) at 12, 24, and 36 hours, respectively, after MPTP treatment compared with saline controls. Validation of differential expression of a subset of genes by quantitative real-time RT-PCR confirmed the Oligo GEArray data. This knowledge of the changes in gene expression levels after MPTP treatment should lead to a better understanding of the mechanisms of neural progenitor cell death or elimination of cells in the SVZ and RMS.

4. MPTP neurotoxicity to neural progenitor cells is protected by MAO-B inhibitors

Neurotoxic effects of MPTP to nigrostriatal dopaminergic system (DA) are thought to be initiated by MPP+, which is a metabolite formed by the monoamine oxidase (MAO) B-mediated oxidation of MPTP. In order to determine whether the same mechanism is involved in the neurotoxicity of MPTP to neural progenitor cells (NPCs), this study looked at the effects of MAO B inhibitor, R(-)-deprenyl (deprenyl) and a classic MAO-A and MAO-B inhibitor, pargyline, both of which protect against MPTP neurotoxicity to DA.

Male C57BL/6 mice were divided into four groups: saline alone, deprenyl (or pagyline) plus saline, deprenyl (or pagyline) plus MPTP and MPTP plus saline. Deprenyl or pagyline (both 30 mg/kg) was intraperitoneally (i.p.) administered 1 hour prior MPTP injection (i.p., 20 mg/kg x 4, 2 hour intervals). Apoptotic cells in the saline- or saline plus MAO-B inhibitor-treated animals were few while MPTP induced marked induction of apoptosis in the SVZ and RMS at 1 d after administrations. When mice were pretreated with a MAO-B inhibitor, deprenyl or

pagyline, not only the nigrostriatal dopamine depletion but also NPC death were significantly protected against MPTP insult. Although deprenyl has also been suggested to posses neuroprotective effects independently, while pargyline has no such effect, implicating the neurotoxicity of MPTP to NPC is also mediated by a mechanism of MAO-B conversion to MPP+. In the current study, the evidence that MPTP-induced NPC apoptosis is also notably found in both postnatal 21 days (PND 21) and 12-month-old mice, suggesting that the apoptotic neurotoxicity of MPTP to NPC is not likely an age-dependent phenomenon.

5. Transiently impaired neurogenesis is a consequence of MPTP-induced apoptosis of neural progenitor cells

In adult mice, newly generated neural progenitor cells (NPCs, neuroblasts) in the SVZ migrate along the rostral migratory stream (RMS) to enter the olfactory bulb (OB), where they differentiate into granule cells and periglomerular interneurons. The present study was thus designed to investigate the effects of MPTP injury on neurogenesis within OB in adult mice. C57BL/6 mice were injected intraperitoneally with bromodeoxyuridine (BrdU) prior to MPTP treatment to detect newborn cells *in vivo* and to follow their fate in the OB. When sacrificed at 2, 7, 14, and 28 days after BrdU labeling and MPTP treatment, mice exhibited a significant decline in the density of BrdU-positive cells in the granule cell layer compared with saline-treated controls. At 14 and 28 days after MPTP injury, neurogenesis, defined by the number of cells with colocalization of BrdU and a neuronal nuclei marker, NeuN, declined significantly in the granule cell layer of the OB. Decreased neurogenesis was accompanied by a reduction in TH-positive interneurons in the periglomerular cell layer (PGL). Precursor cells (C cells) were also found increased at 7 days after MPTP injury using immunohistochemistry and ultrastructural analysis, followed by a recovery of Dcx-positive NPCs at 14 days after MPTP injury.

Taken together, these data demonstrate that besides a reduction in dopaminergic neurons in the substantia nigra, neural progenitor cell death also occurs in the SVZ and RMS in the MPTP mouse model of Parkinson’s disease.

The present findings provide new insight into the extensive neurotoxicity of MPTP and may be valuable in reevaluating the MPTP mouse model of PD. Furthermore, MPTP-treated mice may be used as a new tool to explore the mechanism of differentiation or elimination of neural progenitor cells in the adult SVZ and RMS.