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Lack of CCR5 modifies glial phenotypes and population of the nigral dopaminergic neurons, but not MPTP-induced dopaminergic neurodegeneration

Dong-Young Choi [a,b,c], Myung Koo Lee [b], Jin Tae Hong [b,c,*]

[a] College of Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Republic of Korea
[b] College of Pharmacy, Chungbuk National University, 12 Gaesin-dong, Heungduk-gu, Cheongju, Chungbuk, 361-763, Republic of Korea
[c] Medical Research Center, Chungbuk National University, 12 Gaesin-dong, Heungduk-gu, Cheongju, Chungbuk, 361-763, Republic of Korea

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ABSTRACT

Constitutive expression of C–C chemokine receptor (CCR) 5 has been detected in astrocytes, microglia and neurons, but its physiological roles in the central nervous system are obscure. The bidirectional interactions between neuron and glial cells through CCR5 and its ligands were thought to be crucial for maintaining normal neuronal activities. No study has described function of CCR5 in the dopaminergic neurodegeneration in Parkinson's disease. In order to examine effects of CCR5 on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration, we employed CCR5 wild type (WT) and knockout (KO) mice. Immunostainings for tyrosine hydroxylase (TH) exhibited that CCR5 KO mice had lower number of TH-positive neurons even in the absence of MPTP. Difference in MPTP (15 mg/kg × 4 times, 2 hr interval)-mediated loss of TH-positive neurons was subtle between CCR5 WT and KO mice, but there was larger dopamine depletion, behavioral impairments and microglial activation in CCR5 deficient mice. Intriguingly, CCR5 KO brains contained higher immunoreactivity for monoamine oxidase (MAO) B which was mainly localized within astrocytes. In agreement with upregulation of MAO B, concentration of MPP+ was higher in the substantia nigra and striatum of CCR5 KO mice after MPTP injection. We found remarkable activation of p38 MAPK in CCR5 deficient mice, which positively regulates MAO B expression. These results indicate that CCR5 deficiency modifies the nigrostriatal dopaminergic neuronal system and bidirectional interaction between neurons and glial cells via CCR5 might be important for dopaminergic neuronal survival.

Introduction

Parkinson's disease (PD) is the third most prevalent neurodegenerative disease after Alzheimer's disease and dementia with Lewy body disease (Smeyne and Jackson-Lewis, 2005). The neurodegenerative disorder is pathologically characterized by dopaminergic neurodegeneration in the substantia nigra and consequent depletion of dopamine in the striatum. Until now, specific underlying mechanisms by which the dopaminergic neurons are degenerated in PD brains remain to be elucidated.

The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model has been extensively used since the system recapitulates several cardinal features of PD including dopaminergic neuronal loss in the substantia nigra, depletion of dopamine level in the striatum and impairment in locomotor activity. MPTP-mediated dopaminergic neurotoxicity is elicited by 1-methyl-4-phenyl pyridium (MPP+), an active neurotoxic metabolite of MPTP which is mainly produced by monoamine oxidase (MAO) B in astrocytes (Youdim et al., 2006). Once MPP+ is released from astrocytes, the chemical is taken up by dopaminergic neurons through the dopamine transporter (DAT) leading to the neuronal death. MAO B is a key player in MPTP neurotoxicity as administration of deprenyl, a selective MAO B inhibitor prevents MPTP-induced dopaminergic neurotoxicity (Heikkila et al., 1985; Langston et al., 1984). Evidence showed astroglial activation and increased MAO B activity with aging (Alper et al., 1999). In addition, MAO B activity is elevated in astrocytes around beta-amyloid-positive plaques (Gulyas et al., 2011). These findings suggest that rise in MAO B activity might be related to pathogenesis of neurodegenerative diseases. A recent study showed that overexpression of MAO B in astrocytes causes dopaminergic neurodegeneration (Mallajosyula et al., 2008). In the study, they showed that overexpressed MAO B elevated production of membrane permeant H_2O_2 which could oxidize dopamine within dopaminergic neurons. This was accompanied by mitochondrial complex I dysfunction and increased mitochondrial superoxide resulting in dopaminergic neuronal death.

Abbreviations: PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP+, 1-methyl-4-phenyl pyridium; MAO, monoamine oxidase; DAT, dopamine transporter; CCR, C–C chemokine receptor; MIP, macrophage inflammatory protein; KO, knockout; WT, wild type; MAPK, mitogen-activated protein kinase; DOPAC, dihydroxy phenyl acetic acid; HVA, homovanillic acid; TH, tyrosine hydroxylase; GFAP, glial fibrillary acidic protein.

* Corresponding author at: College of Pharmacy, Chungbuk National University, 12 Gaesin-dong, Heungduk-gu, Cheongju, Chungbuk 361-763, Republic of Korea. Fax: +82 43 268 2732.
E-mail address: jinthong@chungbuk.ac.kr (J.T. Hong).
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Chemokines and their corresponding receptors are broadly expressed in the central nervous system and regulate glial and neuronal cell functions (Asensio and Campbell, 1999). Emerging evidence indicates that chemokine receptors are involved in neuronal death and hence neurodegenerative diseases (Cartier et al., 2005). Among various chemokine receptors, CC chemokine receptor 5 (CCR5) is stimulated by three β-chemokines: CCL3/macrophage inflammatory protein-1a (MIP 1α), CCL4/MIP-1β and CCL5. Constitutive CCR5 expression has been detected in astrocytes, microglia and neurons (Sorce et al., 2011). Although roles of CCR5 in the central nervous system are obscure, bidirectional interactions between neuron and glial cells through CCR5 and its ligands are thought to be crucial for maintaining normal neuronal activities. In support, RANTES attenuated inflammatory response of microglia after motor neuron injury via CCR5, and demise of motor neurons was accelerated in CCR5 null mice (Gamo et al., 2008). Moreover, CCR5 deficient mice seemed to be vulnerable to ischemic injury (Sorce et al., 2010). In the study, ischemic brain injury was induced by occlusion of the middle artery, and resultant motor deficit and infarction were worse in CCR5 knockout (KO) mice than wild type (WT) mice.

We have shown astroglial activation in the brains of CCR5 knockout (KO) mice, which might be mediated by compensatory increase in CCL2-mediated signaling (Lee et al., 2009). CCL2 could activate microglia and astrocytes resulting in activation of p38 mitogen-activated protein kinase (MAPK) (McMahon and Malcangio, 2009), which might upregulate MAO B expression (De Zutter and Davis, 2001; Wong et al., 2002). We hypothesized that CCR5 deficiency might be related to astroglial activation and concurrent increase in MAO B activity. Here, we show that CCR5 KO mice have lower number of the nigral dopaminergic neurons, and worse MPTP-mediated impairments in the nigrostriatal dopaminergic system than WT mice.

Materials and methods

Animals and MPTP treatment

CCR5 KO mice on the C57BL6/J background were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). CCR5 KO mice had no overt developmental abnormalities. We bred CCR5 KO mice with C57BL6/J mice to obtain CCR5 heterozygous mice. Then, male and female CCR5 heterozygous mice were bred to generate CCR5 WT and KO mice. Genotypes of mice were confirmed by polymerase chain reaction. Detection of CCR5 WT allele was achieved by using primers 5′-CAGGCAACAGAGACTCTTGG-3′ (oIMR6916) and 5′-TCATGTTCT CCTGTGGATCG-3′ (oIMR6917). Primers for detection of CCR5 KO allele were 5′-CTTGGGTGGAGAGGCTATTC-3′ (oIMR0700) and 5′-AGGTGAGATGACAGGAGATC-3′ (oIMR0701). The animals were maintained and handled in accordance with the guideline for the humane care and use of laboratory animals of the Korea Food and Drug Administration. All of the experimental procedures were conducted in accordance with the protocol approved by the Institutional Animal Care and Use Committee of Chungbuk National University (approval number: CBNUA-144-1001-01). We used 5–6 month-old male CCR5 KO and WT littermates in this study. Mice were received intraperitoneal injection of MPTP (15 mg/kg, Sigma-Aldrich, St Louis, MO) or saline four times with 1.5 hr interval. We employed the MPTP dose to differentiate MPTP-induced dopaminergic neurotoxicity between CCR5 WT and KO mice. They were sacrificed 7 days after injections for experimental analyses.

Behavioral tests

We performed behavioral tests 6 days after MPTP injection to examine whether there is difference in the neurotoxicant-caused behavioral deficit between CCR5 WT and CCR5 KO mice. Rota rod, pole and gait tests were conducted as following.

Rota rod test

Motor performance and coordination were examined using the Rotarod treadmill (MED Associates Inc., St. Albans, VT.), consisting of a 3.6-cm diameter cylindrical treadmill connected to a computer-controlled stepper motor as described previously (Vijitruth et al., 2006). When the animal falls off the rotating drum, individual sensors sense it, and automatically record the length of time spent on the treadmill (in seconds). Mice were trained two consecutive days before MPTP injections in acceleration mode (2–20 rpm) over 5 min. The training was repeated with a fixed speed (16 rpm) until the mice were able to stay on the rod for at least 150 s. If animals did not pass the trainings, they were excluded from further experiments. On day 6 after MPTP injections, mice were assessed for their performance with a maximum latency of 150 s. Rotational speeds successively accelerated with 16, 20, 24, 28, and 32 rpm and the overall rod performance (ORP) for each mouse was calculated by the trapezoidal method as the area under the curve in the plot of time on the rod versus rotation speed.

Pole test

We conducted the pole test to reflect bradykinesia as described by Okuda et al. with minimal modifications (Okuda et al., 2006). Briefly, a rough-surfaced wooden pole (1 cm in diameter, 55 cm in height) was vertically placed on the floor of home cage. Upon being placed head-upward on top of the pole, mice turned downward and descended back to their home cages. Prior to the test trials, mice were acclimatized to the pole over three trials with intervals of 30 s between the trials. On the test trials, total time for them to orient downward and descend to the floor was assessed. The test trials were performed three times per animal and average values from three examinations were used for each animal.

Gait test

Stride length was measured according to the methods of Fernagut et al. (2002). Briefly, forelimbs and hindlimbs were painted with ink and animals were placed on a bright runway (4.5 cm wide, 42 cm long, with walls 12 cm high) and were allowed to run toward a dark goal box (20 × 17 × 10 cm). Mice were subject to two training trials to be acclimatized at the environment. A single test trial was performed and stride length was measured as the distance between successive paw prints. Data was presented as the average of five strides for each animal.

HPLC analysis of neurochemicals and MPP+

Dopamine and metabolites in the striatum were measured by HPLC. Briefly, tissues were sonicated in chilled 0.1 M perchloric acid containing dihydroxybenzylamine as an internal standard. After centrifugation (15,000 × *g*, 30 min, 4 °C), supernatant was diluted with mobile phase (75 mM of NaH_2PO_4, 1.7 mM Octane sulphonic acid, 10% methanol, pH 3.0) and 10 μl of sample was isocratically eluted through a 80 × 4.6 mm C18 column (Waters Associates, Milford, MA) with flow rate of 1.5 ml/min. Neurochemicals including dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) were detected by a two-channel electrochemical detector (Waters Associates) at a potential of 1.5 mV. Concentrations were normalized by wet tissue weight.

MPP+ concentration was assayed according to a previous study (Jackson-Lewis and Przedborski, 2007). In short, mice received intraperitoneal injection of MPTP (30 mg/kg). Nigral and striatal tissues were dissected 2 h after MPTP injection, and homogenized in 0.4 M $HClO_4$ (100 mg tissue/ml) containing internal standard (4-phenyl pyridine). The mixture was centrifuged at 20,000 × *g* for 30 min at 4 °C. 90 μl of the supernatant was mixed with 10 μl of mobile phase consisting of acetonitrile and 50 mM potassium phosphate adjusted to pH 3.2 with phosphoric acid. The mixture was transferred into HPLC