The Levels of Plasma IL-1β, IL-6 of C57BL/6J Mice Treated with MPTP and Brain Lateralization

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The work is to explore the relationship between the levels of cytokines (IL-1β and IL-6) in C57BL/6J mice treated with MPTP and brain lateralization. By using paw preference test, right-pawed, left-pawed mice models were established. Following single injection of 1-methyl-4-pheny1-2, 3, 6-tetrahydropyrid (MPTP) (40 mg/kg) to impair dopaminergic neuron, enzyme linked immunosorbent assay (ELISA) kits were used for detection of plasma levels of cytokines. The results showed that in saline treated C57BL/6J mice (control), there was no obvious difference observed between left-pawed and right-pawed mice in plasma levels of IL-1β and IL-6. In MPTP treated mice, there was no difference between level of IL-1β in left-pawed mice and that in right-pawed ones in statistics, that is, they were increased on day 1 and day 3, but decreased on day 6. The plasma level of IL-6 was lower in left-pawed than that in right-pawed mice (p<0.005) after MPTP treatment. On day 1 and day 3, the level of IL-6 was almost the same as control; on day 6, it was significantly increased, higher than that of control (p<0.001) in left-pawed mice. While in right-pawed mice, on day 1 and day 3, it was no different from control, too. And on day 6, it significantly increased in compared with control (p<0.005). In conclusion, the level of plasma IL-6 of C57BL/6J mice treated with MPTP increased. The variation of IL-6 was correlated to brain lateralization. *Cellular & Molecular Immunology*. 2004;1(3): 219-223.

Key Words: MPTP, Parkinson's disease, brain lateralization, neuroimmunity, cytokine

Introduction

Brain lateralization is formed during brain development, and it is a universal phenomenon existing in humans and animals. Cerebral lateralization is evidenced on basis of anatomical, neurochemical and functional data. There is widespread agreement that the two sides of brain contribute differently to the regulation of human behavior (1). Through ablation experiment in brains and clinical observation, now it is considered that under physiological conditions the right neocortex depress immune functions, whereas the left neocortex enhance the immune functions. Many previous studies also showed that brain lateralization was involved in the modulation of immune response including cytokine secretion through the asymmetrical neuroendocrine system (2-5). In mice the link between brain lateralization and immune response was first demonstrated using paw preference in a food-reaching task. For its stableness, it is commonly used as a good model of behavior lateralization for research.

Parkinson's disease (PD) is a slow processing movement disorders in the elderly. The molecular mechanism of the neurodegeneration in PD remains enigmatic. So many studies on PD burst out in recent years *via* a variety of PD models. The potent neurotoxin, 1-methyl-4-pheny1-2, 3, 6-tetrahydropyrid (MPTP) is known to selectively damage nigrostriantal dopaminergic neurons, and it is now extensively applied to investigate the pathophysiology and pathogenesis of PD (6). In humans and nonhuman primates, MPTP induced irreversible and severe motor abnormalities almost identical to those in PD. That includes a marked induction in the levels of striatal dopamine and its metobolites. Furthermore, some studies on mice model of PD induced by MPTP, suggested that many proinflammatory cytokines such as IL-1β, IL-2, IL-4, IL-6 and TNF-α were increased in corpus striatum and cerebral spinal fluid (7-9). Whereas, up to date, there are no relevant reports about the interactions between these cytokines and brain lateralization in such animal models of PD. So, the purpose of this report is to study the correlation between the levels of cytokines (IL-1β and IL-6) in mice model of PD and brain lateralization. By using paw preference as an index of lateralization, left-pawed, right-pawed, and ambidextrous mice were initially classified, and subsequently, such male C57BL/6J mice were treated

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Abbreviations: PD, Parkinson's disease; ADH, antidiuretic hormone; RPE, right paw entry; MPTP, 1-methyl-4-pheny1-2, 3, 6-tetrahydropyrid; OVLT, organum vasculosum lamina terminalis; ELISA, enzyme linked immunosorbent assay; HPA, hypothalamic pituitary adrenal.

with MPTP, which can selectively destroy nigrostriantal dopaminergic neurons (6,10) in that animal model with PD was established, and then plasma levels of IL-1β and IL-6 were bound to be detected.

Materials and Methods

Animals

Male C57BL/6J mice (4-week-old, healthy) were purchased from Experimental Animal Center of Chinese Academy of Sciences, Shanghai, China. Upon arrival, the mice were housed five per standard cage and maintained on foods and water ad libitum with a 12-h day/night cycle. They were allowed to adapt to their environment for at least about 1 week before behavioral testing. Behavioral test and final test were all performed at 9 am.

Behavioral procedure

Paw preference was determined according to the method of Colins (11). Mice were deprived of food for 18 h during the night and on the next morning placed in a cubicle (4.5 cm × 4.5 cm × 6 cm) with a centered feeding tube. The mice could reach a pellet of food only with the use of one paw. The right paw entry (RPE) score was determined as the number of RPE that were observed out of 50 reaches per session. A series of four sessions were taken within two weeks. The mean score from the last three sessions was used to determine paw preference classification, the first session being considered as a learning session. Mice were classified as right-pawed if the RPE score was equal to or more than 30, as left-pawed if the RPE score was equal to or less than 20, and ambidextrous if the RPE score was between 21 and 29. One week later, left-pawed and right-pawed mice were divided into 4 groups at random, 5 to 7 mice per group. Because of its fewness, ambidextrous mice were excluded from the experiment and were only used for observing the distribution pattern of C57BL/6J mice.

Establishment of experimental animal models of PD

Left-pawed and right-pawed mice were assigned to two groups, control and experimental groups. In experimental group, MPTP (Sigma) 40 mg was dissolved in 0.5 ml of sterile saline and injected subcutanously to the back of the mouse (12). Controls received an equivalent injection of sterile saline. Mice treated with MPTP began to show static tremor and rigidity from day 2, which became worse on day 6. The animals were killed by decapitation on day 1, day 3, day 6 (received injection of MPTP for 1 day, 3 days and 6 days) .All efforts were made to minimize the number of animals used and their suffering. Trunk blood was rapidly collected in heparinized tubes and then centrifuged at 3000 rpm for 15 minutes. Plasma was stored at –80°C until use.

Detection of IL-1β and IL-6

Enzyme-Linked-Immunosorbent-Assay (ELISA) was used to detect the levels of IL-1β and IL-6. IL-1β ELISA kit (R&D) and IL-6 ELISA kit (Bender) were purchased from the Shenzhen Jingmei Lrd. The procedures were done according to the indications: (1) 50 μl of standard, control buffer, and sample were added respectively into wells of the ELISA plate coated with antibody IL-1β or IL-6, then incubated at room temperature for 2 hours. (2) After that, the wells were washed 5 times and 100 μl HRP was added into each well and incubated for 2 hours. (3) Washed again, 100 μl treated TMB was added into each well, incubated for 30 mins at room temperature in the dark. (4) 100μl 1 mol/L H_2SO_4 was added to stop the reaction. (5) Finally read absorbance at 450 nm by microreader (Bio-Rad, 3550-UV) within 30 minutes of stopping reaction with wavelength correction at 570 nm.

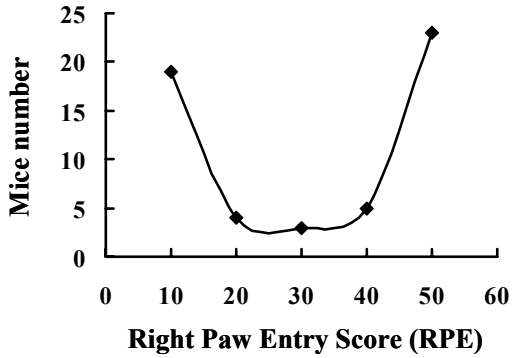

Figure 1. Distribution of paw preference in the population of C57BL/6J mice (RPE*). Paw preference is represented as the RPE scores.

Data analyses and statistic

Statistical analysis of IL-1β and IL-6 was performed by one-way analysis of variance (ANOVA) and Independent-Samples *t* test with the software of EXCEL and SPSS11.0. Differences were considered significant when the *p* value was <0.05.

Results

The distribution pattern in C57BL/6J mice with brain lateralization

54 male C57BL/6J mice were selected. According to RPE score, 23 left-pawed mice, 28 right-pawed mice and 3 ambidextrous mice were included, which was distributed like U-distribution pattern. Among these, RPE score of

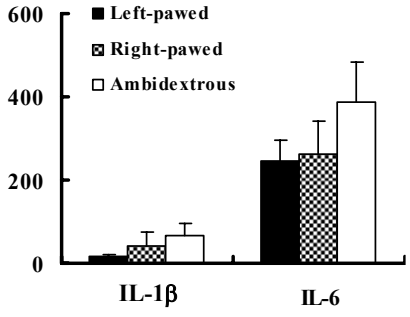

Figure 2. Plasma levels of IL-1β and IL-6 in C57BL/6J mice treated with saline (control) (left-pawed n=5, right-pawed n=7, ambidextrous n=3).

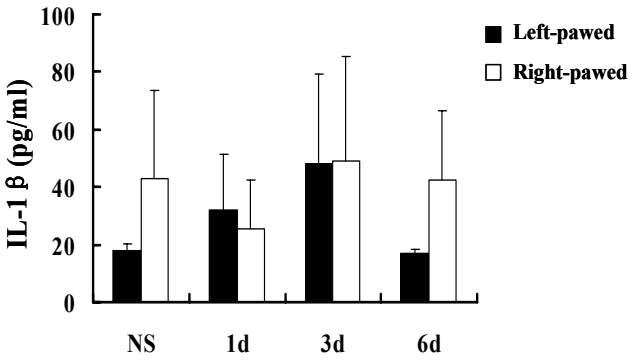

Figure 3. Plasma levels of IL-1β in right-pawed, left-pawed C57BL/6J mice treated with MPTP (left-pawed control n=5, left-pawed MPTP 1d, 3d, 6d group n=6; right-pawed control n=5; right-pawed MPTP 1d, 3d, 6d group n=7).

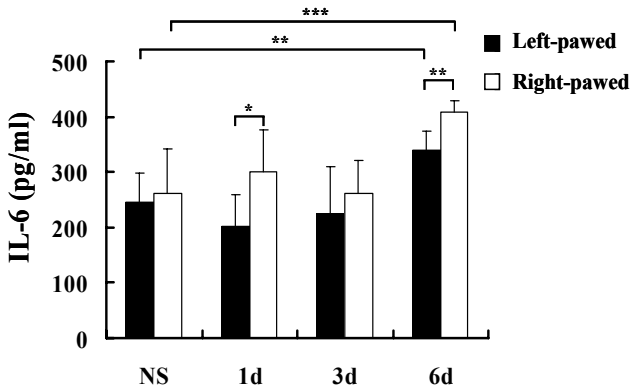

Figure 4. Plasma levels of IL-6 in right-pawed, left-pawed C57BL/6J mice treaded with MPTP (left-pawed control n=5, left-pawed MPTP 1d, 3d, 6d group n=6; right-pawed control n=5; right-pawed MPTP 1d, 3d, 6d group n=7. *p <0.05; **p<0.01; ***p<0.005).

left-pawed mice mainly was centralized in 1-5, while right-pawed mice were in 45-50 (Figure 1).

The plasma levels of IL-1β and IL-6 in saline treated C57BL/6J mice (control)

The results of plasma levels of IL-1β and IL-6 in control group without injection of MPTP showed that: the levels of IL-1β and IL-6 were higher in ambidextrous than that in right-pawed mice, and the levels of those in right-pawed were higher than that in left-pawed, but there was no significant difference in statistics (p>0.05) (Figure 2).

Plasma levels of IL-1β and IL-6 after single injection of MPTP

(1) Plasma level of IL-1β: it increased both in left-pawed and right-pawed mice on day 1 and day 3, while it was decreased on day 6. And the variation of IL-1β in left-pawed was similar to that in right-pawed mice (Figure 3), but there was no statistical significance (p>0.05). (2) Plasma level of IL-6: the level of IL-6 in left-pawed mice was at the same level as control on day 1 and day 3, whereas on day 6, it significantly increased higher than that of control (p<0.01); in right-pawed mice, the level of IL-6 was also at the same level as that of control on day 1 and day 3, and it increased obviously higher than that of corresponding control on day 6 (p<0.005) (Figure 4). (3) After the treatment with MPTP, the variation of IL-6 in right-pawed was much greater than that in left-pawed mice on day1 and day 6, and there was notable statistical significance (p<0.005) (Figure 4).

Discussion

For the sake of establishing animal models for Parkinson's disease (PD), the knowledge of human being on PD was enlarging. So far, the animal models of PD in common use included: intravenous injection of MPTP in rhesus, direct injection of 6-hydroxydopamine to the substantia nigra and corpus striatum in rats and intraperitoneal administration of MPTP to mice as well. Among these models mentioned, the mice MPTP model was a better and widely-used one on account of its appealing hints: namely economical, ample supply, and most importantly, the behavioral and biochemical alterations in this model are extremely the same as real PD (13, 14), so that it may lead to new insights into the pathogenesis of PD. German (15, 16) et al. found that the sensitivity to MPTP varied from species, strains and sexes of the mice. For instance, compared with several different strains of mice, C57BL/6J mice had the highest sensitivity to MPTP, while the same sensitivity of CF-W mice, FVB/N mice and Balb/c mice was a little lower than that of C57BL/6J mice, and the sensitivity to MPTP of CF-1 mice and CD-1 mice is the lowest. Furthermore, among C57BL/6J mice, male C57BL/6J mice adopted in our experiment was the most highly sensitive to MPTP.

In our study, 54 male C57BL/6J mice were selected. According to paw preference test, these 54 mice were divided into three groups: 23 left-pawed mice, 28 right-pawed mice and 3 ambidextrous mice, which was distributed like U-distribution pattern. In other words, left-pawed and right-pawed mice occupied majority proportions while ambidextrous mice occupied minority, that is, the result was in accordance with the previous reports (17). As for cytokines, in saline treated C57BL/6J mice (control), the levels of IL-1 β and IL-6 were higher in ambidextrous than in right-pawed mice and levels in right-pawed were higher than in left-pawed mice. All of these results differed from our previous finding in female Balb/c mice (18), which implied that dissimilar strains and sexes of the mice may contribute to various outcomes in animal experiments. In current studies, male C57BL/6J mice were adopted whereas female Balb/c mice were adopted previously.

It was widely known that brain lateralization encompassing structural and functional asymmetries is a universal biologic phenomenon. In common sense, brain lateralization was defined as the heterogeneities of anatomic structure, transmitter secretion and function in two hemispheres of biological individuals. However, broadly speaking, brain lateralization not only indicated the difference of anatomic structure, transmitter secretion and function of individual brain in the same species, but also meant the functional heterogeneity of other systems resulted from the

brain. A good case in point was handed-ness, which was a good mirror to brain lateralization. In mice, the link between brain lateralzation and immune reactivity was usually demonstrated by using the paw preference as an index of lateralization. On the basis of such asymmetrical animal models, scientists found that the neuroendocrine and immunological characteristics differed in left-pawed, right-pawed and ambidextrous animals. It has been confirmed that there were notable relations between immune responses and behavioral lateralization, and that the activation of the hypothalamic-pituitary-adrenal (HPA) axis observed during the stress in response to a physical stimulus is related to lateralization (19), so there is an association between paw preference and some immune parameters (20). Thus, attribute to such an animal models with brain lateralization, studies of interaction between the neuroendocrinology and immunology could be explored deeply. In Cabib's experiments, he demonstrated that different distribution of dopamine, one of the neurotransmitters in brain, had close correlation with brain lateralization. In other words, the levels of dopamine in the brain differed obviously in left-pawed, right-pawed and ambidextrous mice (21). Moreover, our previous work suggested that: both in LPS-treated and untreated mice, the plasma levels of IL-1β, IL-6, TNF-α, corticosterone, NO were all associated with brain lateralization (22-25). So, since the nervous system had a significant influence on the immune system mostly via the neurotransmitters, we could make a conjecture that, the asymmetry distribution of neurotransmitters in brain may be related to the heterogeneities of plasma levels of cytokines in such asymmetrical animal models. The elevated level of IL-6 in our test, was distinctly higher in right-pawed than that in left-pawed mice, but there were no obvious changes in the plasma level of IL-1β. All these results above indicated that the destruction of dopaminergic neurons by MPTP could affect plasma level of IL-6 but not IL-1β. Considering the facts that IL-6 was mainly generated by monocytes and macro- phages where the dopaminergic receptors also distributed, dopaminergic neurotransimitters were supposed to modulated the secretion of plasma IL-6. Since MPTP acted as a dopamine loss inducer in PD animal model, the variation of plasma IL-6 in our experiment could be found. Moreover, in respect that asymmetrical distribution of dopamine neurons in brain of both right-pawed and left-pawed, synchronously the existent heterogeneities of plasma levels of IL-6 in this study, the asymmetrical distribution of neurotransimitters in brain should be partly responsible for the disaccord of immune response in the lateralized animal models.

Both IL-1β and IL-6 are vital molecules of neuro-endocrine-immune networks, and they can trigger hypothalamic-pituitary-adrenal (HPA) axis, then exert great effects. Meanwhile, the exorbitant activated HPA axis may bring on notable increase of corticotropin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and corticosterone (CS). In the circulation, plasma IL-1β and IL-6 could be transported into the brain via median eminence, organum vasculosum lamina terminalis (OVLT) and choroid plexus, where the blood-brain barrier was absent in these areas. So subsequently, these cytokines could directly initiate biosynthesis and secretion of CRH and antidiuretic hormone (ADH) in hypothalamus (26). Some relevant studies have showed that the levels of IL-1β and IL-6 in plasma were related with the activity of HPA axis (27, 28), nay, whether in patients or animal models of PD, many reports also demonstrated there were always overactivation in HPA axis (29, 30). As to our experiments, the level of IL-1β in MPTP-treated mice did not exhibit changes, whereas the level of IL-6 was elevated greatly. Hence, given the factors above and the results we found, we made a speculation that the activation of HPA axis both in patients and animal models of PD should be mediated mostly by IL-6.

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