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Original Article

Antidepressant efficacy of MLC901 in the 6-hydroxydopamine mice model of Parkinson's disease

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ABSTRACT

Introduction: Depression is a common mood disorder in patients with Parkinson's disease (PD), which negatively influences the quality of life and enhances caregiver burden. MLC901, a traditional medicine, has been demonstrated to be useful in preclinical and clinical studies. The aim was to study the effect of MLC901 on depression behavior in a mouse model of PD, comprising in the unilateral striatal delivery of the neurotoxin 6-hydroxydopamine (6-OHDA).

Methods: Female NMRI mice were divided into the following groups: sham/saline group, 6-OHDA/saline group, sham/MLC901 (40µg/kg) group and 6-OHDA/MLC901 group. Intraperitoneal treatments of MLC901 were started one week after the stereotaxic surgery that continued for 4 weeks (5 days/week). Locomotion was monitored using an open-field test and depressive-like responses were measured by forced swim test (FST) and tail suspension test (TST).

Results: We found that MLC901 prevented the increased immobility time in the PD mice in both FST and TST, suggesting an antidepressant efficacy for the MLC901. None of the treatments alter locomotion compared to the sham group.

Conclusion: In conclusion, we propose that MLC901 is a potential candidate to be used in studies for the treatment of depression in PD.

Keywords:
Parkinson's disease
MLC901
Forced swim test
Tail suspension test

Introduction

Besides motor dysfunction, depression is one of the most typical non-motor syndromes, occurring in around 35% of Parkinson's disease (PD) patients (Reijnders et al., 2008). Along with the progression of PD, depression has been correlated with declined functioning, cognitive disruption and increased stress, which considerably contribute to the substandard quality of life for PD patients (Kadastik-Eerme et al., 2015). Neuroimaging evidence has indicated that depression in PD was the result of re-

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gional abnormalities in the basal ganglia, the prefrontal cortex and the limbic system (the ventral striatum, thalamus, amygdala, insula and cingulate cortex) (Etkin et al., 2010; Remy et al., 2005). Hence, the pathophysiology of depressive symptoms in this disease is complex and probably modulates by the neurotransmitter systems of dopaminergic, serotoninergic, noradrenergic and cholinergic (Kamińska et al., 2017). Though L-DOPA remains the most effective treatment for the motor symptoms of PD, its impacts on mood-correlated dysfunctions is constricted and its long-term application worsens depression (Jaunarajs et al., 2012; Nègre-Pagès et al., 2010). Therefore, new treatment strategies are needed to avoid the onset of depression in PD patients.

Interestingly, traditional Chinese medicine MLC901 has played a critical participation in promoting health and disease control for many years ago in Asia. Its possible therapeutic effect is often ascribed to the synergistic characteristic of numerous herbal constituents which provide a combinational therapeutic strategy which ameliorates the efficacy via hitting numerous targets (Heurteaux et al., 2013; Lorivel et al., 2015; Siow, 2008). Recently, this medicine has come out as a hopeful treatment for ameliorating useful recovery of patients afterward ischemic stroke (Siow, 2008). Protective effects of MLC901 on performance of mice in cognitive tasks has been known (Lorivel et al., 2015). Enhanced hippocampal neurogenesis along with promoted proliferation and neuronal differentiation as well as survival of young neurons has been also shown with MLC901. It is supposed that the neurogenesis efficacy of MLC901 is contributed to its pro-cognitive properties (Lorivel et al., 2015). In a recent study, protective effect of MLC901 on memory in Alzheimer's disease patients has been found (Chen et al., 2019). We previously reported that MLC901 abolishes fear memory impairment in the sleep-deprived rats (Nasehi et al., 2019).

Based on the mentioned document, we wanted to examine the effect of MLC901 administration on depressive-like behavior in mouse model of PD produced via the microinjection of 6-OHDA.

Material and methods

Drugs

The 6-hydroxydopamine hydrochloride (6-OHDA, obtained from Tocris Bioscience) was dissolved in a vehicle solution (0.9% sterile saline comprising 0.02% ascorbic acid). The 6-OHDA dose was chosen based on literature (Alvarez-Fischer et al., 2008). The MLC901 (Moleac, Singapore) combines nine herbal particles with the following composition in each capsule: 0.57g Radix astragali, 0.114g Radix *Salvia miltiorrhizae*, 0.114g Radix *Paeoniae rubra*, 0.114g *Rhizoma chuanxiong*, 0.114g Radix *Angelicae sinensis*, 0.114g *Carthamus tinctorius*, 0.114g *Prunus persica*, 0.114g Radix polygalae and 0.114g Rhizoma acori tatarinowii. Ketamine hydrochloride and xylazine (Alfasan, Woerden, Holland) used for animals anesthetization. Animals received saline (10ml/kg) or MLC901 (40µg/kg, intraperitoneally) for four weeks (5 days/week). The volume of administration was 10ml/kg. MLC901 dose was selected based on (Widmann et al., 2018).

Animals

Female NMRI mice (6–8 weeks old, 28-32g) were kept eight per cage under standard controlled laboratory conditions (12h light/dark cycle, light on at 07:00am, 22±2°C, pelleted food and water ad *libitum*). All experiments performed upon the ethical principles confirmed in the Guide for the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 85–23) as well as approved by the Animal Care and Research Ethics Committee of Tehran Medical Sciences (Islamic Azad University).

Experimental plan

The mice were anaesthetized with a mix of xylazine (10mg/kg) with ketamine (100mg/kg) and positioned in a stereotaxic frame. Mice received bilateral microinjection of 6-OHDA (4µg/µl, 0.5µl/each site) into the dorsal-lateral striatum (coordinates: Anterior-Posterior: +0.9; lateral about the sagittal line: Lateral: ± 1.8; vertically from the top of the skull: Ventral: -3.2). Sham mice were microinjected with the similar volume of vehicle (0.9% sterile saline and 0.02% ascorbic acid). Afterwards, the animals returned to their cages for recovering (one week). A total of 32 mice were divided into four groups (n=8), including: sham group received saline (10mg/kg), 6-OHDA group received saline (10mg/kg), MLC901 in non-PD mice and MLC901 in PD mice. The compounds were tested at doses: saline (10mg/kg), 6-OHDA (4µg/µl) and MLC901 (40µg/kg). MLC901 administrations were started one week after

the surgical procedure for four weeks (5 days/week). Then, 24h afterward the last drug injection, the mice were submitted to the behavioral tests.

Behavioral testing

Open-field test

The open-field apparatus was comprised of white opaque Plexiglas (50×50×30 cm^3). The floor was partitioned to four quadrants of equal part. At the time of test, the device was illuminated by a dim light. Number of crossings with all paws from one part to another as an index of locomotor activity was observed for 5 min (Alijanpour et al., 2019).

Forced swimming test (FST)

The FST was performed 5min after the open-field test. Animals were positioned into a cylindrical container (10cm in diameter, 25cm in height, 10cm in water depth) filled with water and maintained at 23-25°C. Immobility time was measured for the period of the last 4min of the total 6min swimming. Immobility means that animals end swimming and make no active movement (Alijanpour et al., 2019).

Tail suspension test (TST)

The TST was done 1h after the FST. Animals were suspended from the upper of a lever with their tail fixated on a hook via insertion adhesive tape (about 1cm from the tail tip). The immobility time was measured in the course of the last 4min of the total 6min suspension. Immobility means that animals end struggling and stay motionless (Alijanpour et al., 2019).

Data analysis

For statistical analysis, one-way ANOVA followed by Tukey Post Hoc analysis was used for numerous comparisons. The $P<0.05$ was considered significant.

Results

Effect of MLC801 treatment on locomotor activity in PD mice

One-way ANOVA and Post-Hoc analysis exhibited that PD mice tended to decreased locomotor activity [$F(3,28)= 3.01$, $P=0.125$] compared to the vehicle/saline group, although it was not significant. MLC901 did not alter locomotion in vehicle-treated mice or PD mice. As a result, PD mice showed a mild motor impairment.

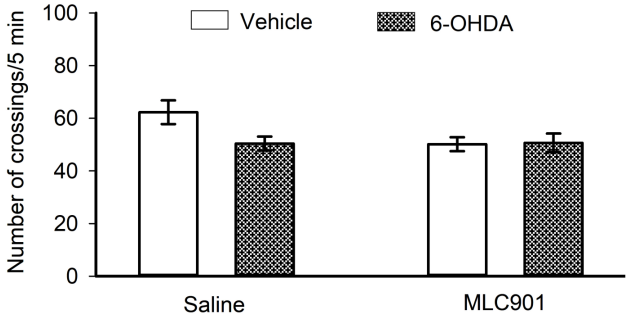

FIGURE 1. Effect of MLC901 on locomotor activity of PD mice in the open-field test. Values are mean±SEM (n=8 in each group). 6-OHDA: 6-hydroxydopamine

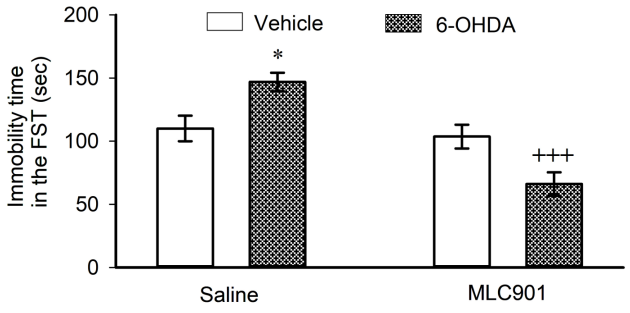

FIGURE 2. Effect of MLC901 on immobility time of PD mice in the FST. Values are mean±SEM (n 8 in each group). $^{*}P<0.05$ compared to the vehicle/saline group; $^{+++}P<0.001$ compared to the 6-OHDA/saline group. 6-OHDA: 6-hydroxydopamine; FST: forced swimming test.

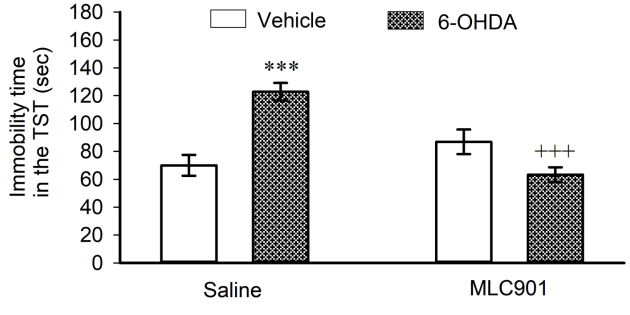

FIGURE 3. Effect of MLC901 on immobility time of PD mice in the TST. Values are mean±SEM (n=8 in each group). $^{***}P<0.001$ compared to the vehicle/saline group; $^{+++}P<0.001$ compared to the 6-OHDA/saline group. 6-OHDA: 6-hydroxydopamine; TST: tail suspension test.

Effects of MLC901 treatment on immobility time in the FST in PD mice

One-way ANOVA and Post-Hoc analysis demonstrated that PD mice significantly increased immobility time [$F(3,28)= 13.171$, $P<0.001$] in the FST compared with vehicle/saline group, but PD mice treated with MLC901 decreased this parameter compared to the PD/saline group. As a result, PD mice showed a depressive-like property but MLC901 could prevent this effect in the PD mice.

Effects of MLC901 treatment on immobility time in the TST in PD mice

One-way ANOVA and Post-Hoc analysis displayed that PD mice enhanced immobility time [$F(3,28)= 13.884$, $P<0.001$] in the TST compared with vehicle/saline group, while PD mice treated with MLC901 decreased this parameter compared to the PD/saline group. As a result, PD mice showed a depressive-like property but MLC901 could prevent this effect in the PD mice.

Discussion

We found that MLC901, a traditional Chinese medicine, prevents depressive behavior and locomotor activity dysfunction in Parkinson's mice of 6-OHDA model. We created the PD model presented by Bonito-Oliva and coworkers (2014). This study showed that the PD model induced by bilateral infusion of 6-OHDA in the dorsal striatum produces a partial reduction (70%) in the dopamine and noradrenaline release (Bonito-Oliva et al., 2014). In line with this study, we observed that PD mice tended to decrease of locomotion in the open-field test, although it was not significant. Moreover, PD mice exerted the depressive-like response in the FST and TST (two standard behavioral paradigms indicative of depression). The 6-OHDA selectively abolishes the dopaminergic nigrostriatal circuit through producing oxidative stress which can cause the production of inflammation and finally cell death (Kaariainen et al., 2008; Lev et al., 2013). In addition, the neurotoxin 6-OHDA rapidly exerts non-enzymatic oxidation which produces superoxide and hydrogen peroxide as well as hydroxyl radicals (Soto-Otero et al., 2000). It seems that 6-OHDA, as a last effector of dopamine neural death, elicits a caspase 3-dependent apoptotic signaling (Hanrott et al., 2005; Tanaka et al., 2006); however, this circuit is not particular for 6-OHDA (Hartmann and Hirsch, 2001). Alvarez-Fischer et al. (2008) indicated that the toxicity of 6-OHDA is partially mediated through α-synuclein because α-synuclein knockout animals are more resistant to 6-OHDA and mechanism of α-synuclein toxicity is very probable mediated through reactive oxygen species production. In an animal model of PD, the production of free radicals may cause a enhance in pro-inflammatory cytokines in company with reduced anti-inflammatory particles in the striatum (Barbiero et al., 2014; Pisanu et al., 2014). There is a report that depression associated with PD is related to an alteration in the neuroinflammation-caused serotonergic system (Santiago et al., 2016).

We observed that treatment with MLC901 for four weeks could prevent depressive-like profile induced in PD mice. Recently, it has been reported that MLC901 prevents inflammatory mechanisms afterward focal cerebral ischemia (Widmann et al., 2018). Several preclinical researches have demonstrated a main beneficial influence of MLC901 on stimulation of ATP-sensitive K^+ channels (Maati et al., 2012), a mechanism revealed to be neuroprotective (Zhang et al., 2016) as well as on the repair mechanism such as neurogenesis or expression of the cortical brain derived neurotrophic factor (Heurteaux et al., 2010). This compound also stimulates serine/threonine kinase Akt (protein kinase B) signaling in model of global ischemia (Franke et al., 1997) and decreases level of the Bax protein as well as induces a reduction of apoptotic mechanisms (Quintard et al., 2011).

Conclusion

The multifaceted positive effect of MLC901 in neuroprotective and neurorepair processes suggests that this compound can be useful for the treatment of depression in PD.

Acknowledgment

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Conflict of interest

There is no conflict of interest in this manuscript.

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