Pharmacological Activation of mGlu4 Metabotropic Glutamate Receptors by TAS-4 attenuates neuroinflammation and dopaminergic neurodegeneration in the MPTP model of Parkinson's disease

Dube Aakanksha, Chaudhary Sumit, Magdum Amol and Upasani Devidas Chandrashekhar

Abstract— Oxidative stress and inflammation play a crucial role in Parkinson's disease (PD) pathogenesis and may represent a target for treatment. Current PD drugs provide only symptomatic relief and have limitations in terms of adverse effects and inability to prevent neurodegeneration.

This study examined whether TAS-4, a selective mGlu4 metabotropic glutamate receptors positive allosteric modulator has neuroprotective effect in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease (PD). Oral administration of TAS-4 (30 mg/kg, PO, BID) for 21 days significantly improved motor coordination on Rotarod and improved grip strength in MPTP-induced mouse model of Parkinsonism. TAS-4 (30 mg/kg, PO, BID) also significantly attenuated dopamine depletion as well as significantly decreases IL-6 level in striatum of MPTP Treated mice. The MPTP-induced neuroinflammation, neurodegeneration and behavioral impairments were markedly repudiated by treatment with TAS-4.

These results suggest that TAS-4 protects dopaminergic neurons from MPTP-induced toxicity in the mouse model of PD.

Key words: Dopamine, Metabotropic Glutamate Receptor 4-Positive Allosteric Modulator (mGluR4 PAM), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), Parkinson's disease (PD), TAS-4.

1 Introduction

Parkinson's disease (PD) is a common chronic progressive neurodegenerative disease. PD is results of the death of dopaminergic neurons in basal ganglia and resulted in symptoms like tremor and bradykinesia [1]. Dopaminergic neuronal loss in the subtantia nigra and dopamine deplection in striatum results in to motor impairemet in PD. The characteristic in PD is presence of degeneration of dopaminergic neurons, glia cell activation and Lewy bodies' formation [2].

Clinically PD is characterized by motor symptoms like resting tremor, Bradykinesia, rigidity and postural instability. Striatal dopamine (DA) level reduction is results from the selective and progressive degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNpc) [3]. Still cause of PD unclear, however, the involvement of oxidative stress, mitochondria the dysfunction and apoptosis is reported. Medication for current therapy for PD is to treat the symptoms of the disease without halting or delaying the degeneration of DAergic neurons [4]. Therefore the neuroprotection is the therapeutic strategy for the PD.

Several evidences and reports were supports role of neuroinflammation in the pathogenesis of PD [5]. Though the mechanisms underlying neurodegeneration is obscure, some progresses have been made in identifying mechanisms (mitochondrial dysfunction, oxidative stress, excitotoxicity & neuroinflammation). The increase oxidative stress is resulted in to the neuroinflammation. Among the environmental factors, pesticides and heavy metals play critical roles in the pathogenesis of PD. Several environmental toxins have been used to develop rodent models to understand the biochemical and molecular mechanisms of PD pathogenesis and therapeutic interventions. 1-Methyl 4-phenyl 1, 2, 3, 6-tetrahydropyridin (MPTP) mouse model has been considered as one of the most accepted models of PD [6]. It was reported that accidental exposure of drug abusers to 1-methyl-4-phenyl-1, 2, 3, 4-tetrahydropyridine (MPTP), an inhibitor of mitochondrial complex I, resulted in an acute and irreversible parkinsonian syndrome. Therefore, MPTP is widely used as a tool to study the molecular events that lead to degeneration of dopaminergic neurons in animal models of PD and to test potential neu-

Dr. Chandrashekhar Devidas Upasani Department of Pharmacology, Faculty of pharmacy, Shri Neminath Jain Bramhacharyashram's Shriman Sureshdada Jain college of pharmacy Jain gurukul, Chandwad, Nashik,423101 Maharashtra,India

Aakanksha Dube. Department of Pharmacology, Faculty of pharmacy, Shri Neminath Jain Bramhacharyashram's Shriman Sureshdada Jain college of pharmacy Jain gurukul, Chandwad, Nashik,423101 Maharashtra,India
dubeaakanksha@gmail.com

roprotective agents [7]. In the light of above findings, in the present study we have attempted to investigate the potential anti-inflammatory and neuroprotective effects of TAS-4 on MPTP-induced mouse model of PD.

2. MATERIALS AND METHODS

2.1. Reagents

Assay kit for IL-6 (R&D system) were purchased. Rotarod (Panlab) and MK-801 & MPTP (Sigma, St. Louis, MO) were purchased.

2.2. Animals

Male C57BL/6 mice weighing 20 to 25 g were used for MPTP study. Mice were housed four per cage in a temperature and humidity-controlled environment with food and water available ad libitum. They were maintained on a 12-h light/dark cycle (lights on 7:00 AM, lights off 7:00 PM) and all studies were conducted between 9:00 AM and 5:00 PM. All behavioural testing was performed between 8:00 AM and 2:00 PM. These procedures involving the use of animals and their care were conducted in conformity with CPCSEA Guidelines. All studies involving animals were approved by Institutional animal ethical committee of SNJB College of pharmacy, Chandwad.

2.3 In vivo pharmacokinetic profile of TAS-4 in mice

Male C57BL/6J, weighing approximately 20 to 25 g, were used for pharmacokinetic studies. The animals were acclimat-ed to their surroundings for approximately 1 week before dos-ing and provided food and water ad libitum. Parenteral administration of compounds to rats was achieved via a penile vein injection at a dose of 3 mg/kg (2 % DMSO + 98% saline, 1 ml/kg, i.v.) and a dose volume of 10 ml/kg. Blood collections via the retro orbital puncture under isoflurane anesthesia were performed at predose and 5, 15, 30, 60, 120, 240, 480 min post dose. Samples were collected into chilled EDTA-fortified tubes and centrifuged for 10 min at 5000 rpm (4°C), and the resulting plasma was aliquoted into 96-well plates for HPLC analysis. Pharmacokinetic parameters were obtained from noncompartmental analysis (WinNonLin, V5.3; Pharsight, Mountain View, CA) of individual concentration time profiles after the parenteral administration of a test article. For systemic exposure studies (Both systemic plasma and central nervous system tissue exposure) TAS-4 (30mg/kg, PO) was administered orally suspended in an aqueous solution of 1% Tween 80 and 0.5 % Carboxy methyl cellulose, q.s., 10 ml/kg, p.o. Blood samples were collected at 5, 15 and 30 min and 1, 2, 4, and 8 h post dose. Whole brain samples were collected at 0.5, 1, 2 and 4 h. Whole blood was collected into chilled EDTA-fortified tubes, centrifuged for 10 min at 5000 rpm (4°C) and stored at -80°C until HPLC analysis. The brain samples were rinsed in phosphate-buffered saline, snap-frozen, and stored at -80°C. Before HPLC analysis, brain samples were thawed to room temperature and subjected to mechanical homogenation by using IKA homogenizer.

2.4 MPTP and TAS-4 treatments

Male C57BL/6 mice were trained on rotarod for 5 days before start of MPTP injections. MPTP induced dopaminergic cell loss was induced by five doses of MPTP (30 mg/kg, s.c.) administered with a 24 h interval. Control mice received a similar injection of saline. TAS-4 (3, 10 & 30 mg/kg, PO, BID) was administered 2 h before MPTP administration and continue for 21 days.

2.4 Behavioral studies

All behavioral tests were performed blindly with respect to drug administration. Animals were randomly divided into different groups. Behavioral studies were repeated with three different trials to validate the behavioral data.

2.4.1 Rotarod

Rotarod was used to evaluate the motor coordination skill of mice of each group. The Rotarod unit consists of a rotating rod, which was divided into four parts by compartmentalization to permit the testing of four mice at a time. After twice daily training for 5 successive days (10 rpm on the first two day and 15 rpm on last three day). for each mouse to remain on the rotating bar was recorded for three trials, at 15 min intervals with maximum trial length of 180 s per trial. The motor deficiency was evaluated as the ability of the mouse to hold the rotating rod.

2.4.2 Grip test

The grip test is used to measure the maximal strength of forelimbs. Briefly, the apparatus consist of a wire of 50 cm length, pulled tight between two vertical supports and elevated 40 cm from a flat surface was used. Mice fore limb were place on a wire and supported until they hold the wire with their forelimbs and then latency to fall was recorded by stopwatch.

2.5 IL-6 levels

Mice striata were homogenized in tissue lysis buffer (50 mMTris–HCl, pH 8.0, 5 mM NaCl, and 1% Triton X-100). Supernatants from homogenates were used for determination of IL-6 with commercial ELISA kits.

2.6 Dopamine estimation

Left striatal tissues were used to detect levels of Dopamine by HPLC with electrochemical detector analysis. Left striatal tissues of 8 animals from each group were homogenized in 0.4 M ice-cold perchloric acid (150 µl/tissue). All homogenates were kept away from light in an ice bath for 60 min. Samples were centrifuged at 12,000 rpm, at 4 °C for 20 min, transferring 120 µl supernatant from each sample to a new tube and then add-

ed 60 µl solution (2 mM potassium citrate, 300 mM potassium dihydrogen phosphate & 2 mM disodium EDTA). The supernatant obtained were filtered with a 0.22 µM Millipore filter and the filtrate was injected into the HPLC system for analysis. The mobile phase contained 110 mM citrate buffer / 100 mM EDTA / 70 mM 1-octanesulfonate sodium solution and 20% (v/v) methanol. Flow rate was 1 ml / min.

2.7 Statistical analyses

Results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was applied to calculate the statistical significance between various groups using GraphPad InStat software. A value of $p < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1 In vivo Pharmacokinetic profile of TAS-4

TAS-4 showed rapid oral absorption (Tmax: 1.0 h) with Cmax of 21.4 µM. Absolute bio-availability was 67 %, showing a good pharmacokinetic profile. Low plasma clearance (approximately 5 % of hepatic blood flow) with elimination half life of 3.6 h. TAS-4 also showed an enhanced central penetration and a total brain-to plasma ratio is approximately 1 after oral administration of a 30 mg/kg dose.

3.2 Effect of TAS-4 on Rotarod and Grip strength test

As assessed by Rota rod, MPTP treated group showed a significant depletion in motor coordination skill as compared to naive group on day 7 onwards (Fig. 1). TAS-4 showed dose dependent improvement in Rotarod and grip strength test.
TAS-4 (30 mg/kg, PO, BID) was found to be effective in significant recovery of motor coordination on day 7, 14 & 21 as compared with vehicle group. On day 7 onwards, a significant decrease in motor strength as measured by grip strength test was observed in MPTP injected groups as compared with naïve group. TAS-4 (30 mg/kg, PO, BID) showed significant improvement in grip strength test as compared with vehicle group on day 7, 14 & 21. (Fig. 2). MK-801 (10 mg/kg, IP, BID) showed significant improvement in grip strength test and motor coordination as compared with vehicle group on day 7, 14 & 21.

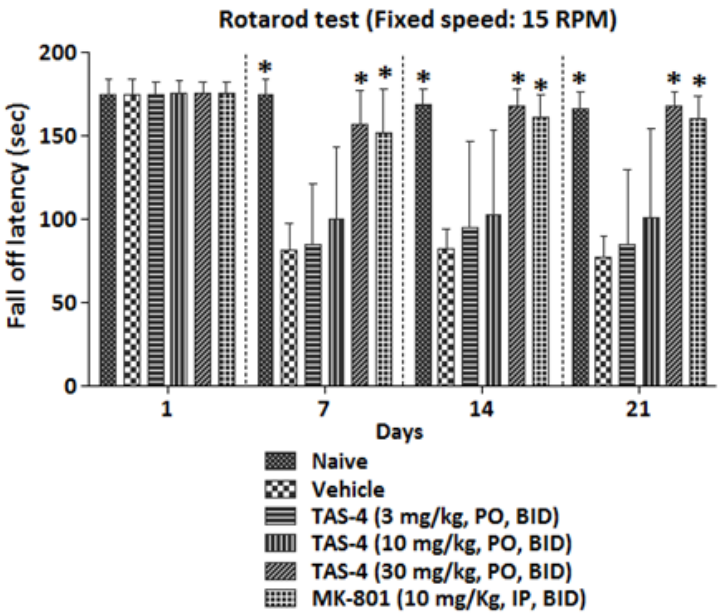

Fig. 1. TAS-4 treatment improves performances in the motor coordination skill. MPTP injection led to significant decrease in motor coordination skill as compared to naive group on day 7, 14 & 21 and significantly recovered in TAS-4 (30 mg/kg, PO, BID) group as compared to vehicle group on day 7, 14 & 21. The data represent as Means ± SEM. (n = 12).

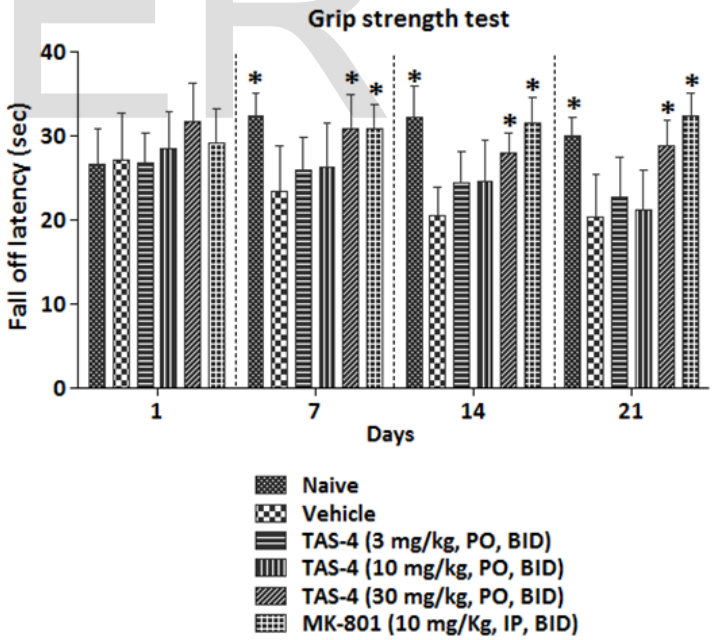

Fig. 2. TAS-4 treatment improves grip strength. MPTP injection led to significant decrease in grip strength as compared to naive group on day 7, 14 & 21 and significantly recovered in TAS-4 (30 mg/kg, PO, BID) group as compared to vehicle group on day 7, 14 & 21. The data represent as Means ± SEM. (n = 12).

3.3 Effect of TAS-4 on striatal IL-6 levels

We analyzed IL-6 protein concentration by ELISA in the homogenate of striatum. As shown in Fig. 3, secretion of inflammatory cytokines were significantly increased at day 21 post MPTP injections when compared with naive group. TAS-4 showed dose dependent decrease in IL-6 level. TAS-4 (30 mg/kg, PO, BID) showed significant decrease in IL-6 level as compared with vehicle group on day 21. MK-801 (10 mg/kg, IP, BID) showed significant decrease in IL-6 level as compared with vehicle group on day 21.

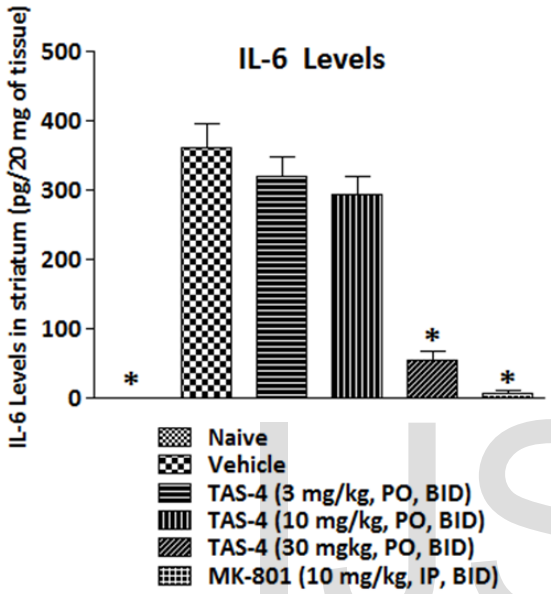

Fig. 3. TAS-4 treatment decreases striatum IL-6 levels. MPTP injection led to significant increase in striatum IL-6 levels as compared to naive group on day 21 and significantly decrease in TAS-4 (30 mg/kg, PO, BID) group as compared to vehicle group on day 21. The data represent as Means ± SEM. (n = 6).

3.4 Effect of TAS-4 on brain dopamine levels

MPTP injections caused significant decrease in the level of dopamine in the striatum of vehicle group as compared to the naive group on day 21. The results in Fig. 4 show that the MPTP-induced dopamine depletion was attenuated in mice treated with TAS-4 (30 mg/kg, PO, BID) for 21 days as compared to the MPTP-injected mice. MK-801 (10 mg/kg, IP, BID) showed significant improvement in dopamine level as compared with vehicle group on day 21.

4. DISCUSSION

MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induces diminution of the dopamine in nigrostriatal pathway and cognitive deficits in mice. MPTP treatment also increases proinflammatory cytokine production in substantia nigra and

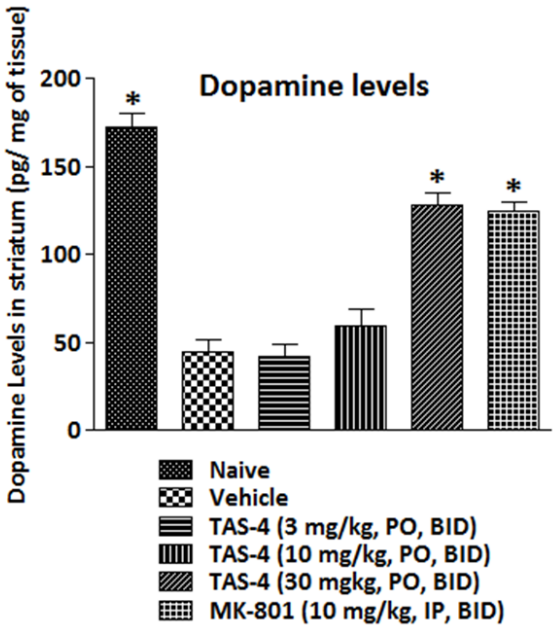

Fig. 4. Effect of TAS-4 treatment on dopamine level in the striata of MPTP injected group mice. The MPTP injections led to a significant decrease in the level of dopamine in MPTP group as compared with the naive group. Treatment with TAS-4 (30 mg/kg, PO, BID) followed by MPTP injection significantly protected the level of dopamine as compared with vehicle group. The data represent as Means ± SEM. (n = 6).

striatum. Since, proinflammatory cytokines influence striatal dopamine content [7].

The MPTP mouse model is widely used for the screening of neuroprotective drugs of potential use in the treatment of Parkinson's disease. MPTP is a lipophilic toxin that crosses the blood–brain barrier and is converted into the active metabolite MPP+ is transported by the DAT into striatal dopaminergic terminals, in which it behaves as a mitochondrial toxin killing nigrostriatal neurons. Endogenous activation of excitatory amino acid receptors is permissive to MPTP neurotoxicity, as originally shown by the protective activity of NMDA receptor antagonists in mice infused with MPP+ in the pars compacta of the substantia nigra [8].

We decided to examine the role of mGlu4 receptors in MPTP toxicity because these receptors are one the most promising targets for symptomatic drugs in experimental parkinsonism. Activation of mGlu4 receptors suppresses GABA release from the striatopallidal terminals of the indirect pathway, thus relieving motor symptoms of parkinsonism [9]. mGlu4 receptor agonists/enhancers meet the requirement to be considered as "disease-dependent drugs" because they appear to act specifically on a pathway that is overactive in Parkinson's disease.

Our finding that TAS-4 protects nigrostriatal neurons against MPTP toxicity adds additional support to the importance of the mGlu4 receptor as a drug target for the treatment of parkinsonism. TAS-4 is a selective positive allosteric modulator of mGlu4 receptors and brain penetrant that has already

shown in our previous work [10].
In this study animals were treated with TAS-4 orally at doses of 3, 10 & 30 mg/kg. TAS-4 at 30 mg/kg, PO, BID showed significant improvent in rotarod and grip strength. Correlation studies demonstrated that the striatal level of IL-6 positively correlated to DA content in striatum. This suggests that IL-6 may have a protective effect on striatal dopaminergic innervation after MPTP. This data is in agreement with the negative correlation between CSF IL-6 levels and severity of Parkinson's disease [11]. Furthermore, using IL-6-deficient mice, it has been shown that this cytokine protects against MPTP- induced denervation, possibly by decreasing microgliosis [12]. Thus, it can be safely concluded that neuroprotection by TAS-4 against MPTP toxicity was entirely mediated by mGlu4 receptors. Whether endogenous activation of mGlu4 receptors is protective against MPTP toxicity is unclear.
In the present study, we showed that mGlu4 receptor enhancers are excellent candidates as novel drugs in the treatment of experimental parkinsonism and encourage additional studies with TAS-4 alone or in combination with L-DOPA in primate models of parkinsonism.

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