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Research article

Modulation of sphingosine-1-phosphate receptor ameliorates harmaline-induced essential tremor in rat

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HIGHLIGHTS

- Sphingosine-1-phosphate analogue typically ameliorated harmaline induced tremor.
- FTY affected explorative and gait disturbances induced by harmaline.
- FTY improved impairments of anxiety-like behaviors following harmaline administration.

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ABSTRACT

Essential tremor (ET) is one of the most common movement disorders with unknown etiology. Despite lack of effective clinical treatments, some potential therapeutic factors and modulation of some neurotransmitters have been utilized to ameliorate motor symptoms in the animal models of tremor. In the current study, male Wistar rats (n = 10 in each group) weighing 40–60 g were divided into vehicle control groups (saline or DMSO), saline/DMSO + harmaline (30 mg/kg, i.p.) + fingolimod (FTY720) (1 mg/kg, i.p, 1 h before harmaline injection) groups. Open field, rotarod, wire grip and foot print tests were used to evaluate motor function. The results demonstrated that administration of FTY720 can improve harmaline-induced tremor in rats. Moreover, FTY720 ameliorated gait disturbance. The results showed that FTY720 can recover step width, left and right step length; however, FTY720 failed to recover mobility duration. FTY720 also improved falling time and time spent in wire grip and rotarod, respectively. The current study provides the first evidence for the effectiveness of FTY720 on motor function in the harmaline model of ET. Furthermore, neuroprotective effects of FTY720 demonstrated in this study offer sphingosine-1-phosphate receptor (S1PR) modulators as a potential neuroprotective candidate against substance-induced tremor and a possible strategy for the treatment of patients with tremor.

1. Introduction

Tremor is characterized by involuntary, rhythmic and sinusoidal oscillation in one or more body parts [2,41]. Essential tremor (ET) as the most common type of tremors and a prevalent movement disorder in adults [33] has negative impacts on many aspects of the patient's life [41].

Despite the fact that ET is one of the most common movement disorders [26], its etiology is still unknown [1]. Animal models of tremor have been largely contributed to better understandings to assess possible pathways involved. Harmaline as an indole alkaloid found in the seeds of *Peganum harmala* (Syria rue) is a widely used tremorigenic compound to experimentally induce tremor in animals [34]. Although the underlying mechanisms of inducing tremor have not been yet clearly elucidated [28], harmaline-induced tremor can be considered as a model to assess the pathogenesis of ET and evaluate possible treatment modalities for this condition [8]. Harmaline acts on the olivo-cerebellar system [29,44], leaving its impacts on the inferior olive nucleus (ION), and can generate tremor by increasing neuronal excitability [25]. Therefore, compounds that are potentially capable of reducing neuronal excitability might be beneficial in the treatment of ET [37].

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Fingolimod (FTY720) is a fungus metabolite [5] that has been proven to be useful in the treatment of inflammatory diseases by exerting immunological and neurobiological effects in the central nervous system (CNS) [23]. FTY720 as an immunosuppressive drug was approved in 2010 for relapsing forms of multiple sclerosis (MS) to attenuate its clinical exacerbation frequency [24]. FTY720 is phosphorylated by sphingosine kinase into its active form, (FTY720-P) in vivo [16]. Neuroprotective effects of FTY720-P in the CNS, as well as FTY720's ability to inhibit not only inflammatory mediators but also T cells have been proposed to contribute to its pharmacological mode of actions [17]. In fact, FTY720 has shown neuroprotective, anti-apoptotic, and immunomodulatory effects in many animal models of CNS-related diseases such as cerebral ischemia, MS and Huntington's disease [6,10].

The goal of this study was to investigate whether behavioral recovery could be pharmacologically enhanced by administration of FTY720 in the animal model of tremor. Here, harmaline induced moderate tremor which was manifested by marked deficits in performance in all behavioral tasks employed, and then these marked deficits were reversed by use of a S1PR modulator.

2. Methods and materials

2.1. Animals

Male Wistar rats (40–60 g) were used. All procedures in this experiment were carried out according to the Neuroscience Research Center of Kerman Medical University. The animals were maintained on a 12-h light-dark cycle with food and water ad libitum.

2.2. Drug preparation and administration

Harmaline hydrochloride dihydrate (Sigma, Germany) was dissolved in normal saline at the concentration of 30 mg/kg. FTY720 purchased from Sigma-Aldrich (Germany) was dissolved in dimethyl sulfoxide (DMSO; maximum DMSO concentration: 1%$^{v}/_{v.}$) at the concentration of 1 mg/ml on the day of experiment. The FTY720 group received FTY720 (1 mg/kg; i.p.), 1 h prior to harmaline injection. Animals which only received harmaline without FTY720 pre-administration, received either saline or DMSO 1 h prior to harmaline injection to keep the same number of injections in all groups. Drugs were administered intraperitoneally to a maximum total injection volume of 1 ml.

2.3. Behavioral tasks

2.3.1. Observation

The occurrence of tremors was rated by an observer who was blinded to the treatment. Thirty minutes after harmaline administration, during the open field test, the tremor data were acquired and quantitatively scored as follows: 0: No tremor, 1: occasional tremor affecting only the head and neck, 2: intermittent (occasional tremor affecting all body parts), 3: persistent (persistent tremor affecting all body parts and tail), 4: severe (persistent tremor rendering the animal unable to stand and/or walk) [3].

2.3.2. Open field test (OFT)

In this test a Plexiglas arena (90 [W] × 90 [L] × 45 [H] cm) was used that its floor was divided into 16 squares to define central and peripheral regions. Each rat was placed in the center of the field. Vertical (rearing) and horizontal activities were recorded for 5 min and analyzed with subsequent offline analysis (Ethovision 7.1, Noldus Information Technology, Netherland). For each rat, total distance moved (TDM, cm); total duration mobility (s) and immobility (s) were recorded. At the end of each trial, the field was cleaned with ethanol 70% [1,20].

2.3.3. Rotarod performance test

In this study, for the analysis of motor coordination and balance skills, accelerating rotarod (Hugo Sachs, Germany) was used. Prior to placing the animal on the apparatus, rod rotation was set to 10 rpm. Upon start of the test, the animal was placed on the rod with the linear acceleration rate of 10 rpm/min to a maximum of 60 rpm. All the animals were pre-trained prior to the experiment and underwent three trials accompanied by a 300-s cut off and inter-trial intervals of 5 min [20,36]. The duration for which each animal remained in the apparatus was recorded and the mean for all trials per animal was calculated.

2.3.4. Wire grip test

This test evaluates muscle strength and balance. To perform the wire grip test, each rat was suspended with forepaws on a horizontal steel wire (80 cm long, 7 mm in diameter), which was connected between two platforms. While rats were grasping the wire, they were placed in a vertical position. All the animals underwent three trials with 5 min inter-trial interval, and the falling latency was recorded with a stop watch [40].

2.3.5. Footprint

To assess the walking patterns and gait kinematics of the animals, footprint test was used. The hind paws of the rats were marked with a non-toxic ink, and then each rat was allowed to walk along a clear Plexiglas tunnel (100 cm [L] × 10 cm [H] × 10 cm [W]), leading to a darkened cage. A sheet of white absorbent paper (100 cm × 10 cm) was placed on the floor of the runway [7]. The distance between each step on the same side of the body at a right angle was measured as stride lengths and the distance between the centers of the respective paw prints to the opposite side of the body as hind paw stride widths. Footprints at the beginning and end of each run were not considered in the analysis [42].

2.4. Statistical analysis

Graph Pad Prism 6 (Graph Pad Software, USA) were used for statistical analysis of data and figure production. All data were first assessed for normality using a Kolmogorov-Smirnov test. Results found to be normally distributed were analyzed using one-way ANOVA test. Where a main effect was seen in ANOVA tests, pairwise comparisons between groups were then made using Tukey's post-hoc tests. Results that were not normally distributed were analyzed using Kruskal-Wallis test. All data expressed as median and interquartile range and $p < 0.05$ was considered statistically significant.

3. Results

3.1. The effects of FTY720 on gait disturbance

Harmaline induced a significant and persistent tremor that affected all body parts. The tremor scale score increased in the rats of harmaline treated compared to the control groups (Fig. 1A) and hind paw stride width also significantly increased (Fig. 1B). Median tremor score and hind paw stride width were significantly decreased by FTY720 treatment compared to the saline + harmaline and DMSO + harmaline groups. These results demonstrate that treatment with 30 mg/kg harmaline reliably induced severe tremor associated with significant functional deficits that can be detected and assessed using the tasks employed. Moreover, a main effect of the treatment upon left (Fig. 1C) and right (Fig. 1D) step lengths was not detected.