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Research report

Myricitrin induces antidepressant-like effects and facilitates adult neurogenesis in mice

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HIGHLIGHTS

- Myricitrin repeated treatment results in antidepressant-like effects in mice.
- Myricitrin increases cell proliferation and the number of newborn neurons in the hippocampal dentate gyrus.
- Myricitrin facilitates differentiation of progenitor cells in neurons in the hippocampus.

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ABSTRACT

Myricitrin (MYR) is a natural flavonoid that inhibits nitric oxide (NO) transmission and has an atypical antipsychotic-like profile in animal models. Considering that several NO inhibitors exert antidepressant-like effects, the present study evaluated the antidepressant-like effect of MYR (3–30 mg/kg) in the tail suspension test (TST). Because of the putative relationship between adult neurogenesis and antidepressant activity, we also assessed cell proliferation, survival, and differentiation in adult neurogenic niches, including the subgranular zone (SGZ) and subventricular zone (SVZ). Similar to the positive control imipramine (IMI; 10 mg/kg), repeated treatment with 10 mg/kg MIR but not acute treatment reduced immobility time in the TST, indicating an antidepressant-like effect. No effect on general motor activity was observed. Myricitrin also facilitated cell proliferation in the SGZ of the hippocampal dentate gyrus and SVZ. In the SGZ, MYR increased the number of doublecortin- and 5-bromo-2′-deoxyuridine/neuronal nuclei-positive cells. Our results suggest that MYR facilitates hippocampal neurogenesis, which might contribute to its antidepressant-like effect and atypical antipsychotic-like profile.

1. Introduction

Myricetin-3-*O*-α-rhamnoside (myricitrin [MYR]) is a naturally occurring flavonoid that is extracted from the genera *Eugenia* and *Pouteria* and has numerous pharmacological effects. Myricitrin exerted antiinflammatory [1] and antioxidant actions in both in vitro and in vivo models [2–5]. Moreover, MYR exerted antinociceptive effects in acute and chronic pain [6–8], anxiolytic-like effects in mice [9], anti-maniac-like effects in rats [10], and neuroprotective effects in an experimental model of Parkinson's disease [5]. Pereira et al. showed that MYR blocked apomorphine-induced climbing and stereotypy and impaired hindlimb retraction time in mice without inducing catalepsy, indicating an atypical antipsychotic-like profile for this compound [11]. The possible mechanism of action of the atypical antipsychotic-like effect of MYR includes nitric oxide (NO) inhibition, in which its behavioral effects were reversed by pretreatment with the NO precursor L-arginine.

Atypical antipsychotics have been shown to exert better antidepressant effects than typical antipsychotics with regard to reducing depressive symptomatology in schizophrenia and are used to augment the treatment of refractory depression [12]. cs also had antidepressant-like effects in rodents that were exposed to the unpredictable chronic mild stress model of depression [13–15]. Although the therapeutic relevance remains speculative, the antidepressant effects of atypical antipsychotics have been hypothesized to be mediated by an increase in adult neurogenesis [16].

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In adult mammals, dynamic changes in cell proliferation and neurogenesis have been shown to occur in two neurogenic regions: the subventricular zone (SVZ) of the lateral ventricles and subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) [17]. Antidepressant drugs and lithium are used to treat mood disorders and have been shown to increase cell proliferation and perhaps promote the subsequent survival of hippocampal neurons, indicating that increased hippocampal neurogenesis may be a common action of antidepressants [18–20]. Non-antidepressant psychotropics, such as haloperidol and opioids, have no effect on or even decrease hippocampal neurogenesis [18,21,22]. Curiously, in the case of the atypical antipsychotics olanzapine, risperidone [16], clozapine [23], and ziprasidone [22,24], increased cell proliferation has been observed in both neurogenic regions (i.e., the hippocampal SGZ and SVZ).

Myricitrin has an atypical antipsychotic profile and inhibits nitric oxide synthase. Nitric oxide inhibitors have been shown to have antidepressant-like properties [25–27] and may stimulate adult neurogenesis in rodents [28]. However, the effects of MYR on depressive-like behavior and adult neurogenesis remain to be investigated. Therefore, the present study investigated the behavioral and pro-neurogenic effects of single and repeated treatment with MYR. Using immunohistochemistry to detect Ki-67- and doublecortin (DCX)-positive cells, we examined the effects of repeated MYR treatment on cell proliferation and neurogenesis, respectively, in the SVZ and SGZ. Using 5-bromo-2′-deoxyuridine (BrdU) as a marker of DNA synthesis, we evaluated the effects of MYR on subsequent cell survival. To confirm the phenotype of the newly generated cells, we used multiple immunolabeling with antibodies against BrdU and neuronal nuclei (NeuN) and glial fibrillary acidic protein (GFAP) markers.

2. Materials and methods

2.1. Animals

One hundred thirteen male Balb/C mice were used (30–35 g, 45 days old). The animals were housed in groups of 5–10 per cage and maintained under conditions of controlled temperature (22 ± 1 °C) with a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The animals received standard commercial chow and tap water ad libitum. All of the experimental procedures were approved by the local ethics committee of the State University of Maringá (CEEA 8488121214) and were in accordance with the guidelines of the U.S. National Institutes of Health and Brazilian College for Animal Experimentation. All efforts were made to avoid the animals' stress and suffering.

2.2. Drugs

Vehicle (saline with 1% Tween 80), imipramine (IMI; 20 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), and MYR (3, 10, and 30 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) were prepared daily immediately before use and injected intraperitoneally (i.p.) in a volume of 1 ml/kg. The MYR doses were based on Pereira et al. [11]. The IMI dose was chosen because it has been shown to reduce immobility in the forced swim test (FST) and TST and increase hippocampal neurogenesis after repeated administration in mice [29–31].

2.3. Experimental design

The mice were randomly assigned to the treatment conditions and tested in a counter-balanced order. The behavioral evaluation was performed between 8:00 AM and 11:00 AM Before the start of the behavioral tests, the animals were allowed to acclimate to the testing rooms for at least 1 h.

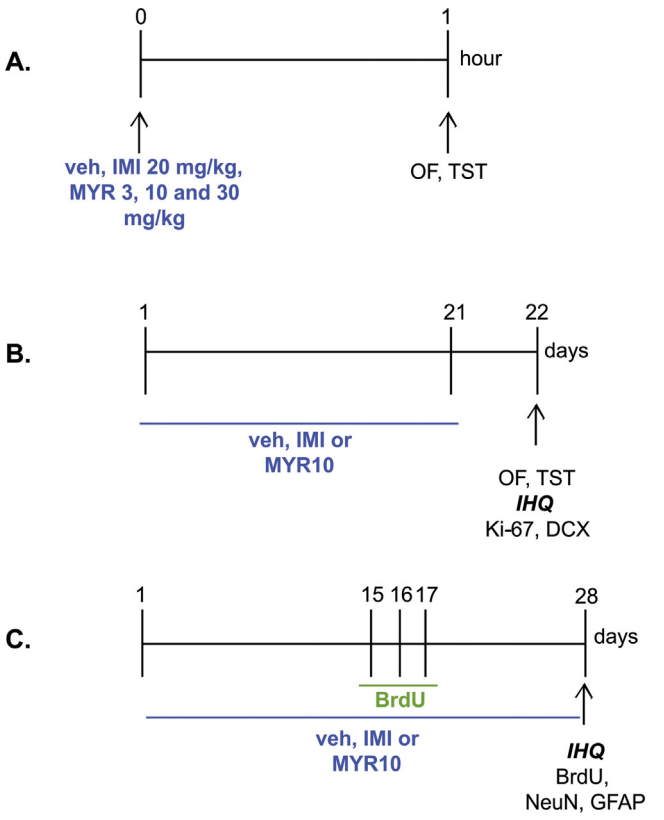

Fig. 1. Experimental design. A) Single i.p. injection of vehicle, IMI (20 mg/kg), or MYR (3, 10 and 30 mg/kg) and behavioral testing. B) Repeated i.p. injection of vehicle, IMI (20 mg/kg), or MYR (10 mg/kg), once daily during 21 days. Behavioral testing was performed 24 h after the last drug administration. C) Analysis of cell survival and maturation after repeated MYR treatment. IMI, imipramine; MYR, myricitrin; OF, open field; TST, tail suspension test; IHC, immunohistochemistry.

The mice received a single i.p. injection of vehicle, IMI (20 mg/kg), or MYR (3, 10 and 30 mg/kg). One hour later, they were evaluated in the open field (OF) and then in the TST (Fig. 1A). To test the effects of repeated administration, vehicle, IMI (20 mg/kg), or MYR (10 mg/kg) was administered i.p. once daily for 21 days. The behavioral testing after repeated treatment was performed 24 h after the last drug administration (Fig. 1B). After the behavioral tests, the animals were perfused under deep anesthesia. Their brains were processed for immunohistochemical analyses to detect DCX- and Ki-67-positive cells (Fig. 1B). For the analysis of cell survival and maturation after repeated treatment, the mice received three i.p. injections of BrdU (100 mg/kg) for 3 consecutive days, starting on day 15 after beginning drug treatment. The animals were euthanized 11 days after the last BrdU injection (day 28). Their brains were then removed and processed for immunofluorescent staining (BrdU, BrdU/NeuN, and BrdU/GFAP; Fig. 1C). All of the behavioral and histological analyses were performed by experimenters who were blind to the experimental groups/treatments.

2.4. Behavioral testing

2.4.1. Open field

To measure locomotor activity and anxiety-like behavior, the open field (OF) test was used [32]. The OF was made of a wooden square box (70 cm × 70 cm) with a 40 cm high wall. The floor was divided into two fields: periphery (20 cm adjacent to the walls) and centre (30 cm^2). The mice were individually placed in the centre of the OF and allowed to explore the box for 10 min. After each session, the OF was cleaned with 70% ethanol and water and then dried. The distance traveled (in meters), number of entries into the centre, and