Psychiatry Research 257 (2017) 249–259

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

A novel flavanone derivative ameliorates cuprizone-induced behavioral changes and white matter pathology in the brain of mice

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ARTICLE INFO

Keywords:
Flavanone derivative
Cuprizone
Schizophrenia
Demyelination
Oligodendrocytes
Neuroinflammation

ABSTRACT

Recent studies have shown that white matter lesions play an important role in the pathogenesis of schizophrenia. DHF-6 is a novel flavanone derivative synthesized in our laboratory. The purpose of the present study was to investigate the effects of DHF-6 on behavioral changes and white matter pathology in a 0.2% cuprizone-fed C57BL/6 mice model. The results showed that cuprizone induced a decrease in spontaneous alternations in the Y-maze test, an increase in locomotor activity in the open field test, demyelination determined by electron microscopy, a decline in the expression of myelin basic protein (MBP), a decrease in the differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes (OLs), and an activation of microglia and astrocytes in the corpus callosum measured by western blot and/or immunocytochemical analyses. Intragastric administration of DHF-6 (25 and 50 mg/kg) for 5-weeks increased the spontaneous alternations, reduced locomotor activity, reversed demyelination and MBP decrease, promoted OPCs differentiation into mature OLs, and inhibited the activation of microglia and astrocytes. These results suggest that DHF-6 may improve cognitive impairment and the positive symptoms of schizophrenia by alleviating white matter lesions *via* facilitating remyelination and inhibiting neuroinflammation, thus may be beneficial in the treatment of schizophrenia.

1. Introduction

Schizophrenia is a common and severe psychiatric disorder characterized by a failure of self-related information processing. Its symptoms include behavioral disorder, negative emotions, and cognitive impairment (Millan et al., 2016). At present, there are two influential hypotheses regarding the disorder, which theorize that dopamine hyperactivity and glutamate hypofunction are involved in the pathogenesis of schizophrenia. These hypotheses are the basis for the use of typical and atypical antipsychotics in the treatment of schizophrenia (Howes et al., 2015). Recently, a number of neuropathological and neuroimaging studies in patients with schizophrenia have shown obvious white matter pathology (Cookey et al., 2014; Lee et al., 2016; Pasternak et al., 2015). There is an association between schizophrenia and microglial activation and proliferation, particularly in white matter (Keller et al., 2013; Kirkpatrick and Miller, 2013; Monji et al., 2009). Given the fact that white matter plays an important role in cognition (Epstein et al., 2014), and since there is as yet no therapeutics that significantly improve cognitive impairment in schizophrenic patients, a new strategy to improve cognitive impairment in these patients by restoring white matter damage may be feasible.

Cuprizone (biscyclohexanoneoxalyldihydrazone) is a copper chelator used as a reagent for copper analysis. Numerous studies have shown that administering a low dose of cuprizone to mice induces consistent demyelination and mature oligodendrocyte loss (Nakajima et al., 2016; Shao et al., 2015). Mice exposed to cuprizone for 4–6 weeks, which is when demyelination and myelin breakdown appear, demonstrate less social interaction (Makinodan et al., 2009), spend more time in the open arms of an elevated plus-maze (Serra-de-Oliveira et al., 2015), and have spatial working memory impairments (Adilijiang et al., 2015). These abnormal behaviors are reminiscent of some schizophrenia symptoms observed in human patients. Thus, cuprizone-treated mice have been used as a novel animal model of schizophrenia in the exploration of the role of white matter abnormalities in the pathogenesis of this mental disorder.

DHF-6 is a novel flavanone derivative (Fig. 1) designed and synthesized in our laboratory (Gu et al., 2017). Our previous studies showed that DHF-6 decreased dopamine D_2 receptor activity and

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http://dx.doi.org/10.1016/j.psychres.2017.07.075
Received 22 April 2017; Received in revised form 4 July 2017; Accepted 30 July 2017
Available online 31 July 2017

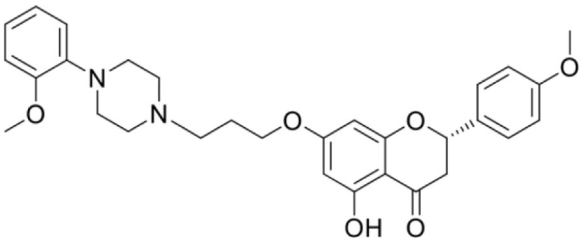

Fig. 1. Structure of compound DHF-6.

inhibited neuroinflammation *in vitro*, and reversed movement hyperactivity induced by MK-801 (an NMDA receptor antagonist) and apomorphine (a dopamine receptor agonist) in mice (Gu et al., 2017). We have found that some natural flavonoids (such as Epimedium flavonoids) ameliorated neuropathological changes in C57BL/6 mice exposed to cuprizone (Liang et al., 2015). In the present study, we investigated the effects of DHF-6 on behavioral impairments, demyelination and neuroinflammation in the brains of mice that underwent cuprizone-induced demyelination.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice weighing 20 ± 2 g (6 weeks old, from Vital River Laboratory Animal Technology Co. Ltd., Beijing, China) were housed under a 12 h light/12 h dark cycle at a constant temperature of 22 ± 1 °C and a relative humidity of 55–60%, with free access to food and water. All experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of Xuanwu Hospital of Capital Medical University, China.

2.2. Drugs and reagents

DHF-6 was synthesized as described previously (Gu et al., 2017) and the purity was over 95% as determined by a high-performance liquid chromatography (HPLC) assay. Clozapine (CLZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). DHF-6 was diluted in 2% Tween-80 in saline, while CLZ was diluted in 2%-Tween 80 in saline supplemented with 30 μL of 0.1 M hydrochloric acid (pH was adjusted to a value close to neutrality, as necessary). Cuprizone was purchased from Sigma-Aldrich (St. Louis, MO, USA), and mixed into the milled Lab diet rodent chow with a final concentration of 0.2% (w/w).

2.3. Experimental manipulations

C57BL/6 mice were randomly assigned to the following 5 groups, each containing 9–10 animals: normal control, cuprizone alone, cuprizone + DHF-6 at low dose (25 mg/kg), cuprizone + DHF-6 at high dose (50 mg/kg), and cuprizone + CLZ (10 mg/kg, positive control drug). The doses of DHF-6 selected were based on our previous study (Gu et al., 2017).

Mice in the control group were fed normal mouse chow, while the groups treated with cuprizone were fed mouse chow containing 0.2% cuprizone (w/w) for 5 weeks. DHF-6 and CLZ were intragastrically administered to cuprizone-treated mice once a day for 5 weeks, while control mice and cuprizone alone group were given only 2%Tween-80 saline water (vehicle). Behavioral tests were carried out on the last day of the 5-week period.

2.4. Y-maze task

The Y-maze apparatus consisted of three arms. Each arm was 35 cm long, 25 cm high and 10 cm wide. Arms were labeled A, B, and C, and were positioned at equal angles. The apparatus was placed 40 cm above the floor and was surrounded by various extra-maze cues. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The sequence of arm entries was recorded (*e.g.,* ABCBAC). An actual alternation was defined as a series of entries into all three arms on consecutive occasions. Therefore, the maximum alternation was the total number of arm entries minus two. The percentage of alternation was calculated using the formula: (actual alternations/maximum alternations) × 100%. The total number of arms entered during the sessions was also recorded (Adilijiang et al., 2015).

2.5. Open field test

Mice were placed in Plexiglas boxes (41.5 cm × 41.5 cm × 41.5 cm) equipped with a video-based Ethovision System (Noldus, Wageningen, The Netherlands) for the evaluation of locomotor activity. Each mouse was recorded for 10 min at 1-min intervals. The total distance traveled by each mouse was calculated (Fernandez et al., 2006; Hoyos et al., 2014).

2.6. Transmission electron microscopy

The ultrastructure of myelinated axons and myelin sheaths was detected by transmission electron microscopy (TEM) according to a previous method (Yin et al., 2012). After the behavioral tests, 3 mice in each group were transcardially perfused with 2.5% glutaraldehyde and 4% paraformaldehyde in PBS (pH 7.4) under deep anesthesia induced by chloral hydrate (400 mg/kg, intraperitoneal injection, i.p.). After removal of the brain from the skull, large blocks of approximately 1 mm^3 were cut from the corpus callosum and postfixed overnight at 4 °C in the same fixative used for the perfusion. The right and left hemisphere were sampled randomly. Blocks were fixed in 1% osmium tetroxide for 35 min. The specimens were dehydrated by gradient alcohol from 50% to 100%, and an embedding reagent was used to replace the water within the tissue. After dehydration and embedment, semi-thin sections (1 μm in thickness) were observed under a light microscope for trimming. Serial ultra-thin sections (50 nm in thickness) were prepared, stained with lead citrate and uranyl acetate, and observed under a Hitachi H-7650 transmission electron microscope (Tokyo, Japan). From each section, ten fields of vision were systematically randomly photographed at a magnification of 15,000×. The first field of vision was randomly selected in the upper left field and the rest fields of vision were captured in a “zigzag” fashion by moving the field using an equidistance movement (Xiu et al., 2015). The ratio of demyelinated axons in total axons (%) was calculated.

2.7. Immunohistochemical staining

After the behavioral tests, 3–4 mice in each group were transcardially perfused with 0.1 M phosphate buffered saline (PBS, pH 7.4), followed by 4% paraformaldehyde in PBS under deep anesthesia induced by chloral hydrate (400 mg/kg, i.p.). Brains were removed and post-fixed in the same fixative at 4 °C overnight, followed by cryoprotection in 30% sucrose at 4 °C for 24–48 h. Serial coronal sections (30 μm) of the brain were cut using a sliding microtome (Thermo Fisher Scientific Corp., Fairlawn, NJ, USA) and collected in 6-well plates containing 0.01 M PBS.

For immunohistochemical staining, free-floating sections (Bregma 0.50 mm) were pre-treated with 3% hydrogen peroxide in PBS for 30 min at 37 °C, washed three times with PBST and incubated for 30 min at 37 °C in a blocking solution composed of 0.3% Triton X-100% and 5% normal rabbit or goat serum. The sections were subsequently incubated in blocking solution containing primary antibodies to myelin basic protein (MBP) (1:2000; Santa Cruz Biotechnology, CA, USA),