Behavioural Brain Research 317 (2017) 147–156

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Umbelliferone reverses depression-like behavior in chronic unpredictable mild stress-induced rats by attenuating neuronal apoptosis via regulating ROCK/Akt pathway

Qin Tingting, Fang Fang, Song Meiting, Li Ruipeng, Ma Zhanqiang**, Ma Shiping*

Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Nanjing 210009, PR China

HIGHLIGHTS

- Umbelliferone ameliorates CUMS-induced depressive-like behavior.
- Umbelliferone protects neurons by preventing neuronal apoptosis.
- The antidepressant-like effects of umbelliferone are achieved by regulating ROCK/Akt pathway.

ARTICLE INFO

Article history:
Received 27 July 2016
Received in revised form
11 September 2016
Accepted 15 September 2016
Available online 16 September 2016

Keywords:
Umbelliferone
Chronic unpredictable mild stress
Depression
Neuronal apoptosis
ROCK/Akt pathway

ABSTRACT

There is increasing evidence that major depressive disorder (MDD) is also a progressive neurodegeneration disorder and neuronal damage is the major pathology of MDD. Umbelliferone, a coumarin derivative, was found in a range of plants with proved anti-oxidative, anti-inflammatory and neuroprotective effects. The primary purpose of this investigation was to evaluate whether umbelliferone could confer an antidepressant-like effect on the depressive model in rats developed by chronic unpredictable mild stress (CUMS) and explore the possible mechanism involved in its neuroprotective effects. We found that treatments with umbelliferone (15 mg/kg, 30 mg/kg) significantly ameliorated CUMS-induced depressive-like behaviors, such as decreased sucrose consumption, reduced locomotor activity and prolonged immobility time. Rats under CUMS stimulation treated with umbelliferone (15 mg/kg, 30 mg/kg) showed reduced neuronal apoptosis, as well as inhibited inflammatory cytokines levels by down-regulating Rho-associated protein kinase (ROCK) signaling and up-regulating protein kinase B (Akt) signaling. In conclusion, umbelliferone showed neuroprotective effects on CUMS-induced model of depression, which was associated with the inhibition of neuronal apoptosis modulated by ROCK/Akt pathway.

1. Introduction

Major depressive disorder (MDD), characterized by a pervasive and persistent low mood, is associated with enormous personal suffering on individuals as well as their families [1]. With a prevalence of 21%, MDD brings a great medical and economic burden throughout the world [2]. However, a number of the clinical trials of depression reflect that present treatments have the limited effectiveness. Many drugs have high rates of partial responsiveness or non-responsiveness, delayed and side effects partly due to unclear mechanisms of the disease [3]. Therefore, it is necessary to study the mechanisms underlying depression for more efficient clinical treatments.

Recent research and neuroimaging studies support the notion that depression can be viewed as a mild neurodegenerative dis-

Abbreviations: ROCK, Rho-associated protein kinase; Akt, protein kinase B; PTEN, phosphatase and tensin homolog; GSK3β, glycogen synthase kinase 3β; CUMS, chronic unpredictable mild stress; CMC-Na, carboxymethyl cellulose; IL-1β, interleukin 1β; IL-6, interleukin 6; TNF-α, tumour necrosis factor α; ELISA, enzyme-linked immunosorbent assay; Umb, umbelliferone; Fas, fasduil; SPT, sucrose preference test; OFT, open field test; FST, forced swimming test.

* Corresponding author at: Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Tongjiaxiang 24, Nanjing, Jiangsu 210009, PR China.
** Corresponding author.
E-mail addresses: mazhanqiang0427@163.com (Z. Ma), spma@cpu.edu.cn (S. Ma).

http://dx.doi.org/10.1016/j.bbr.2016.09.039
0166-4328/

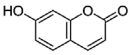

Fig. 1. Structural formula of umbelliferone.

order. According to the studies from basic research and clinical imaging reports, the limbic brain regions, including the prefrontal cortex, hippocampus and amygdala, do have some alterations in mood disorders [4,5]. Postmortem studies have reported that subjects with MDD exhibit a reduced sized pyramidal neurons and a decreased number of GABAergic interneurons [6]. Significant reductions of gray matter in anterior cingulate cortex, amygdala and hippocampus also have been demonstrated by neuroimaging research, which means a degenerative process may happen in MDD [7]. In addition, when rodents and nonhuman primates exposed to chronic stress, many of these responds also occurred, such as atrophy of dendrites and spines in the prefrontal cortex and hippocampus, and decreased neurogenesis in the adult hippocampus [8–10]. Herein, we believe it is possible that the neuronal and glial decrements can be reversed by treatment.

Rho-associated protein kinase (ROCK) is one of the best-characterized downstream effectors of Rho, a member of the small-molecular-weight GTPase superfamily [11]. There are two isoforms of ROCK, ROCK1 (or ROKβ) and ROCK2 (or ROKα). ROCK1 is mostly distributed in non-neuronal tissue, while ROCK2 is strongly expressed in the brain and the spinal cord [12,13]. Recent studies showed the potential therapeutic effects of ROCK inhibitor and ROCK signaling in the treatment of neurodegenerative diseases, such as Alzheimer's disease [14,15], Parkinson's disease [16,17], Huntington's disease [18,19], Amyotrophic lateral sclerosis [20] and Spinal muscular atrophy [21,22]. Likewise, inhibiting ROCK overexpression is beneficial to prevent neuroinflammation and further neuronal damage [23]. Increasing evidence showed that ROCK signaling may be involved in the pathogenesis of psychiatric disorders. Administration of ROCK inhibitor played an important role in mediating anxiety-related behaviors in a mice experiment [24]. Treatment with ROCK inhibitor could cause antidepressant-like effect in rats [25]. In addition, ROCK activity could influence the survival of the neuronal cells, and those pathways involved may be phosphatase and tensin homolog (PTEN) and Akt acting upstream of neuronal survival [26,20]. Therefore, ROCK signaling might be a promising target for depression.

Umbelliferone or 7-hydroxycoumarin (Fig. 1), widely found in many plants such as carrot, coriander and garden angelica [27], is a derivative of coumarin. It has been reported that umbelliferone can be administered safety with nontoxic at a low doses, and cross the blood-brain barrier [28]. Moreover, research studies over the past decades indicated that umbelliferone played an essential role in the regulation of the inflammatory response [29,30]. And umbelliferone treatment has been found to display the neuroprotective effects owning to its potential action on inhibiting apoptosis [31]. Activation ROCK pathway was also found to aggravate inflammation and increase the expression of the inflammatory mediators IL-1 and TNF-α [32]. And ROCK signaling has been revealed to be involved in fundamental cellular functions such as adhesion, migration, proliferation, and apoptosis. However, few reports have focused on the effects of umbelliferone on the activation of the ROCK pathway in the model of depression. In this study we investigated antidepressant-like effect of umbelliferone using a well-established animal depression model of chronic unpredictable mild stress (CUMS), and studied the psychological stress-induced neuronal degeneration and ROCK signaling. Meanwhile, neuroinflammation is found to contribute to the neuronal damage in CUMS model of depression [33,34]. Therefore, we examined behavioral changes, apoptosis-related proteins bax, bcl2 and caspase3 and inflammatory cytokines levels. Furthermore, in order to explore the possible mechanisms underlying the neuroprotective effects of umbelliferone in CUMS rats, we also measured the expressions of ROCK2, phosphorylation of PTEN, Akt and glycogen synthase kinase-3β (GSK3β).

Table 1
The stressors of a CUMS procedure.

Stressor	Duration
Water deprivation	12 h
Food deprivation	12 h
Tilted cage (45°)	12 h
Wet cage	12 h
Illumination overnight	12 h
Foreign object exposure	12 h
Noise	8 h
Forced swimming at 4 °C	5 min
Physical restraint	5 min

2. Materials and methods

2.1. Animals

Healthy male Sprague-Dawley (SD) rats weighing 180–200 g were obtained from the Experimental Animal Center in Jiangsu Province, Nanjing, China. Upon arrival, rats were group housed under standard housing conditions for 7 days (room temperature 25 ± 2 °C; a 12/12 h light/dark cycle) with free access to food and water. We strictly performed all procedures and animal experiments in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation, which were approved by the Science and Technology Department of Jiangsu Province. A concerted effort were made to reduce the number of animals used in the experiment and their suffering.

2.2. Drugs and regents

Umbelliferone (Umb, purity 98%), supplied by National Institutes for Food and Drug Control (Beijing, China), was dissolved in 0.3% sodium carboxymethyl cellulose (CMC-Na). Fasudil (Fas, a selective ROCK2 inhibitor) was purchased from Selleck Chemicals (Houston, TX, USA). Interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) enzyme-linked immunosorbent assay (ELISA) kits were produced by Nanjing KeyGEN Biotech. CO., Ltd. (Nanjing, China).

2.3. Chronic unpredictable mild stress (CUMS) and experimental design

After the adaptation, rats were randomly assigned to six groups as follows (n = 9 each group): Control group, Control + umbelliferone (30 mg/kg) group, CUMS model group, CUMS + umbelliferone (15 mg/kg) group, CUMS + umbelliferone (30 mg/kg) group and CUMS + Fas (10 mg/kg) group. The CUMS procedure was conducted according to a method described previously [35]. To make the procedure unpredictable, the protocol were randomly scheduled and changed every week. All stressors are shown in Table 1.

The experimental procedure for CUMS is shown in Fig. 2. After the rats were exposed to CUMS for 21 days (from 1 st to 3rd week) except for the control rats, they were received treatments respectively for next 21 days (from 4th to 6th week) during which the CUMS stimuli continued. Umbelliferone was administered intragastrically once a day, while fasduil was administered intraperitoneally. All drugs were administered in a volume of 10 ml/kg body weight. For the normal control groups and the CUMS