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Senegenin exerts anti-depression effect in mice induced by chronic unpredictable mild stress via inhibition of NF-κB regulating NLRP3 signal pathway

Hongwei Li, Shiyi Lin, Tingting Qin, Hongyan Li, Zhanqiang Ma*, Shiping Ma**

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

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ABSTRACT

Depressive disorder is a kind of affective disturbance disease. Emerging evidence has suggested that inflammation may contribute to the pathologic process of depressive disorder. Senegenin (SEN), a major bioactive constituent in *Polygala tenuifolia Willd*, has much bioactivity including anti-inflammatory and neuroprotection effects. However, the mechanism of its anti-depressant effect in mice remains unknown. This study aimed to explore the anti-depressant effects of SEN on behavioral changes and inflammatory responses in mice induced by chronic un-predictable mild stress (CUMS). SEN treatment remarkably ameliorated CUMS-induced behavioral abnormalities, such as improving locomotor activity, decreasing immobility time in Tail suspension test (TST) and Forced swimming test (FST), and increasing sucrose intake in Sucrose preference test (SPT). Additionally, SEN improve protein levels of Brain-derived neurotrophic factor (BDNF) and Neurotrophin-3 (NT-3) expression.

In response to stress, p65 was activated to promote production of pro-IL-1β, and then cleaved to mature IL-1β by NOD-like receptor protein 3 (NLRP3) inflammasome pathway in hippocampus of CUMS mice. After SEN treatment, protein activation related to NLRP3 inflammasome pathway was down-regulated, which inhibited IL-1β secretion. These results demonstrate that SEN plays an important role in treatment CUMS-induced depression in mice, possibly via suppression of pathway activation associated with NLRP3 inflammasome.

1. Introduction

Depression is a common major depressive disorder (MDD) with pathologic changes of brain and dystrophy of body. Main clinical presentations of MDD have anorexia, hypothermia, pessimistic and a series of depressive-like behaviors [1]. The 2012 World Health Organization reports that 350 million people are suffered from depression, which will become the second leading disease by 2020 [2]. In clinical treatment of anti-depression, some depression patients are resistant to currently available antidepressants [3]. Moreover, plenty of clinical reports imply that anti-depression drugs have certain side effects, which even surpass the therapeutic effect [4]. Consequently, it is crucial to seek new therapeutic method against depression.

Accumulating evidence shows that brain inflammation may be involved in the development of depression [5,6]. Several clinical studies indeed report the concentration of peripheral pro-inflammatory factors such as IL-1β, TNF-α of depression patients were significantly higher than patients being treated and pro-inflammatory factors were increased in hippocampus and cerebrospinal fluid of depression patients [7–9]. Furthermore, in animals, it has been shown that depressive-like behaviors were induced by pro-inflammatory cytokines and cytokine inducers such as lipopolysaccharide and chronic unpredictable mild stress (CUMS) [10,11]. Pro-inflammatory cytokines can lead to cellular damage and neural plasticity injure in the hippocampus, which is associated with alterations neurotrophic factors signaling, such as brain-derived neurotrophic factor (BDNF) or neurotrophins-3 (NT-3). BDNF and NT-3 help to support the survival and differentiation of existing neurons. Multiple studies have shown that CUMS can reduce BDNF and NT-3 levels in the hippocampus [12]. Accordingly, reducing the level of pro-inflammatory cytokines and inhibition of the neuro-inflammatory as target can exert antidepressant effects.

The cytokines can be regulated by NF-κB and NOD-like receptor

Abbreviations: SEN, senegenin; Flu, fluoxetine; MDD, major depressive disorder; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; CUMS, chronic unpredictable mild stress; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophins-3; NLRP3, NOD-like receptor protein 3; ASC, apoptosis-associated speck-like protein containing; NF-κB, nuclear factor kappa B; SPT, Sucrose preference test; OFT, open field test; FST, Forced swimming test; TST, Tail-suspension test; *i.g.*, intragastrical

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

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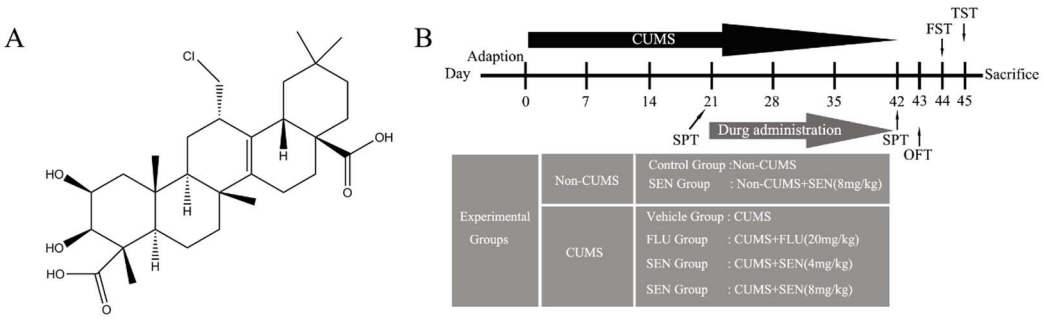

Fig. 1. The construction of Senegenin (A) and Schematic illustration of the experimental procedure (B).

Table. 1
The stressors of a CUMS procedure.

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

protein 3 (NLRP3) inflammasomes pathway. NF-κB can promote the secretion of pro-inflammatory cytokines [13]. NLRP3 inflammasomes belongs to the NLR family and consists of NLRP3, apoptosis-associated speck-like protein containing (ASC), caspase-1 and it is a component of the innate immune system and is responsible for activation of inflammatory progress [14]. At present, NLRP3 inflammasome pathway is a new target in depression [15]. Additionally, the pro-inflammatory cytokines are mainly produced by microglia in brain and the resident macrophage in the central nervous system. The NLRP3 is activated occurrence of oligomerizes in microglia and recruits pro-caspase-1, leading to the activation of caspase-1 and the production of IL-1β [16]. In addition, Papyrus 75 (p75) is a receptor of BDNF and can interact with all neurotrophins including NT-3. When the p75 receptor is activated, it leads to activation of NF-κB receptor [17]. In view of the above, regulating inflammatory reaction could be accepted as an intriguing target for possibly bringing a new approach for anti-depressant therapies.

Senegenin (SEN) (Fig. 1A), a triterpenes and native compound, is from *Polygala tenuifolia Willd* herbal of Chinese medicine. Modern pharmacology research has reported that SEN has anti-inflammatory, anti-oxidant, immunomodulatory and neuro-protective effects, which promotes the differentiation and the proliferation of neural stem cells [18]. Its neuro-protective and anti-inflammatory activity have been extensively recognized [19]. For example, SEN has been proven to have neuroprotective effects against amyloid β-protein induced neurotoxicity in PC 12 cells and attenuates Hepatic Ischemia-Reperfusion Induced Cognitive Dysfunction [20,21]. However, the mechanism of anti-inflammatory effects in mice depression has not been elucidated.

In this study, we investigated the effects of SEN treatment on the depression. Our data indicated that SEN significantly decreased inflammatory responses via inhibition of NF-κB regulating NLRP3 signal pathway during CUMS-induced mice depression.

2. Materials and methods

2.1. Materials

Senegenin (purity ≥ 98%) was purchased from Tianjin SILAN Technology Co., Ltd. (Tianjin, China). Fluoxetine (Flu) was purchased from Changzhou Siyao medical technology Co, Ltd. (Changzhou, China). Enzyme-linked immunosorbent assay (ELISA) kits (TNF-α, IL-1β) were supplied by R & D Systems, Inc. Minneapolis, MN. Bicinchoninic acid (BCA) protein assay was obtained from Beyotime Institute of Biotechnology Co., Ltd.

2.2. Animal treatments and groups

Male ICR Mice, weighting 18–22 g, were bought from College of Veterinary Medicine Yangzhou University (License: SCXK (SU) 2012–0004, Jiangsu Province, China). The quality of the experimental animals review is Institute of Health and Environmental technology, Soochow University (Jiangsu Province, China). The diagrammatic experimental procedure for CUMS is shown in Fig. 1B. The CUMS Mice were randomly divided into six groups (n = 12), including control group, SEN group (8 mg/kg, intragastrical (*i.g.*)), vehicle group (CUMS), Flu group (CUMS, 20 mg/kg, *i.g.*) and SEN group (CUMS, 4 and 8 mg/kg, *i.g.*). To make the procedure unpredictable, the protocol were randomly scheduled and changed every week. All stressors are shown in Table 1. It would take at least 7 days for all mice's adaption to cage environment before the experiment started in a remained at 25 ± 1 °C, day and night in each half with enough food and water. All procedures such as mice feed and care were implemented strictly in agreement with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.3. Behavioral tests

2.3.1. Sucrose preference test (SPT)

The sucrose preference test was performed respectively on 21st and 42nd day at the end of the first SPT. All mice induced by CUMS were randomized into 4 groups. The test was performed as described previously with minor modifications [22]. All mice were deprived of food and water for 12 h before sucrose preference experiment test. The mice were given a free choice of two bottles (one was suffused with a sucrose solution (1% w/v), and the other was suffused with water). After 6 h, replace the positions of two bottles in the cage to avoid potential side-preference effects on drinking behaviors. After 24 h of the experiment, the consumption of water and the sucrose solution were measured by weighing the bottles, then calculate the total intake of liquids and sucrose preference rate. The calculation formula of preference for sucrose = sucrose intake / (sucrose intake + water intake) × 100%.

2.3.2. Open field test (OFT)

In order to evaluate the effects of SEN on spontaneous locomotor activities, we used the OFT as previously described to assess [23]. The apparatus (40 × 60 × 50 cm) was divided into 12 equal squares and the number of crossings was recorded. First of all, the mice were placed in the center of the field adapting to the field environment for 2 min. The test events including the number of crossings (number of line crossings with all four paws), rearings (number of times an animal stood on its hind legs) and groomings (licking, cleaning and scratching its face with the forepaws) were recorded for 4 min. Floor surfaces and