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Research report

Gastrodin ameliorates depression-like behaviors and up-regulates proliferation of hippocampal-derived neural stem cells in rats: Involvement of its anti-inflammatory action

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HIGHLIGHTS

- We investigated early intervention effects of gastrodin on CUS rats' behaviour.
- It reduced depressive-like behaviour and promoted hippocampal and stem cell proliferation.
- It also inhibited NF-κB protein expression and IL-1β level in the hippocampus.
- Moreover, it protected the viability of NSCs from IL-1β-induced damage.
- The findings support gastrodin as a newly antidepressant.

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ABSTRACT

Gastrodin (GAS), an active constituent of the Chinese herbal medicine tianma, has antidepressant-like activity in animals but no specific molecular mechanisms have been identified. In the present study, chronic unpredictable stress (CUS) was used to establish a rat depression model; The sucrose preference test, forced swim test and Morris water maze test were used to assess depression-like behaviors (anhedonia, behavioral despair, motor retardation, and poor spatial memory), and the proliferation of hippocampal stem cells was tested by BrdU immunohistochemistry. The stress and inflammatory responses were assayed by measuring IL-RA, NF-κB, and p-iκB expression by Western blot and IL-1β production by ELISA. Direct and indirect effects of GAS on NSC viable cell number were examined *in vitro* by WST-1 and BrdU assays. It was found that GAS (200 mg/kg daily) reversed all tested depression-like behaviors in CUS model rats and up-regulated NSCs proliferation in the hippocampus. Enhanced expression of p-iκB, NF-κB, and IL-1β by CUS was also reversed by GAS. Moreover, *in vitro* experiments revealed that GAS alone did not increase the viability of NSCs but protected them from IL-1β-induced damage. These results support the antidepressant and neuroprotective effects of GAS, and GAS may reduce depression-like behaviors by protecting hippocampal NSCs against the proinflammatory cytokine IL-1β.

1. Background

According to the World Health Organization, depression will rank as the second most common cause of disability by 2020. Antidepressant drugs are the most common first-line treatments for depression. Current pharmacological treatments, including monoamine oxidase inhibitors (MAOI), tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and noradrenaline-serotonin specific antidepressants (NaSSAs) are generally effective,

Abbreviations: GAS, Gastrodin; CUS, chronic unpredictable stress; NSCs, neural stem cells; SPT, sucrose preference test; FST, forced swim test; MWM, Morris water maze; BrdU, Bromodeoxyuridine; NF-κB, nuclear factor-κB; IL-1R1, IL-1 receptor type 1; IL-1RA, IL-1 receptor antagonist; p-iκB, phospho-inhibitor of kappa B.

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but up to one-third of patients may still show limited response, relapse [1], or drug intolerance [2]. Depression comorbid with another psychiatric disorder such as anxiety may also limit treatment response [3]. Addition of atypical antipsychotics is one treatment option to enhance response rates, but such polypharmacy is also associated with decreased compliance due to side effects [4]. Treatments such as deep brain stimulation (DBM), ECT, or repetitive transcranial magnetic stimulation (rTMS) may be effective for some of these drug-refractory patients, but invasiveness (DBM), low tolerability (ECT), or modest efficacy (rTMS) limit broad application of these techniques [5]. Thus, improved pharmacotherapies are still required to substantially reduce the global burden of major depression.

The development of traditional antidepressants was based on the theory that depression arises from a deficit in brain monoamines or monoaminergic transmission [6]. There is a growing consensus, however, that this theory is insufficient to explain the therapeutic effects of antidepressants [7–9]. Indeed, while these drugs alter synaptic levels of monoamines almost immediately, clinically significant effects are generally not observed for several weeks to months, suggesting that these agents trigger gradual changes in neurochemistry, synaptic transmission, postsynaptic signaling, protein expression, and (or) structural neuroplasticity. The inflammatory and neurodegenerative hypothesis of depression has been formulated gradually [10,11] in parallel with preclinical studies from both animals and humans demonstrating neurodegeneration and reduced neurogenesis in depression caused, at least in part, by inflammation and cell-mediated immune activation. It has even been proposed that depression evolved as part of a complex system of immune defenses against environmental pathogens. Animal models have shown that depression-like behaviors are associated with cell-mediated immune responses [12]. Clinically depressed patients manifest higher levels of inflammatory biomarkers, while proinflammatory cytokines induce neuropsychiatric symptoms as well as major depressive episodes [13,14]. Animal studies have found that IL-1β expression by hippocampal neural progenitor cells and the nuclear factor-κB (NF-κB) signaling pathway are critical for the anti-neurogenic and depression-like behaviors caused by acute and chronic stress [15]. These observations identify neurogenesis and neuroinflammation as potential targets for a newer generation of clinical antidepressants.

Gastrodia elata Blume, known as tianma in Chinese, is a plant of the orchidaceae family, the tuber of which can be dried and used medicinally. According to theories of traditional Chinese medicine (TCM), tianma acts to pacify the liver and "calm wind". Pharmacological studies indicate that tianma has anti-convulsive, sedative, analgesic, nootropic, and anti-inflammatory effects, and improves microcirculation and general circulatory functions; in addition, it is often used in TCM for the treatment of cardio-, cerebro-, and neurovascular diseases including migraine, epilepsy, and depression [16–19]. The phenolic glucoside gastrodin (GAS) is a main active constituent of tianma. It is reported that GAS could inhibit expression of inducible NO synthase, cyclooxygenase-2 and pro-inflammatory cytokines in cultured LPS stimulated microglia [20]; In addition, our previously studies showed that GAS could ameliorates the depressive-like behaviors of CUS rats [21], and it could reverse the levels of IL-6 and IL-1β, and the expression of iNOS and the p38 MAPK phosphorylation in the hippocampus of enhanced single prolonged stress-induced posttraumatic stress disorder rats model [22], suggesting that GAS may suppress specific signaling pathways associated with the immune response, thereby maintaining hippocampal function and preventing behavioral signs of depression. To test this possibility, we investigated the potential neuroprotective efficacy of GAS and its effects on stress related factors NF-κB and IL-1β in the chronic unpredictable stress model of depression. We also observed the effects of GAS on NSC proliferation in the hippocampus, as reduced NSC proliferation is known to occur in depression while antidepressant treatments reverse this effect [23–25].

2. Materials and methods

2.1. Animals

Eight-week-old male Sprague–Dawley rats were obtained from the Fourth Military Medical University animal center and maintained under standard laboratory conditions (12-h light/12-h dark cycle with lights on at 8:00 AM, 22 ± 2 °C with a relative humidity at 50 ± 10%, food and water ad libitum). The animals were allowed to adapt to laboratory conditions for at least one week. All stress-exposed rats were singly housed while non-stressed rats were grouped four/cage. All procedures were in strict accordance with the guidelines established by the US National Institutes of Health (NIH) and approved by the Fourth Military Medical University Animal Care Committee.

2.2. Experimental procedures

After the adaptive phase, rats were randomly divided into 8 groups of 12: sham, sham + GAS (*L*) (low-dose, *L* = 50 mg/kg/day), sham + GAS (*M*) (medium dose, *M* = 100 mg/kg/day), sham + GAS (*H*) (high-dose, *H* = 200 mg/kg/day), CUS, CUS + GAS (*L*), CUS + GAS (*M*), and CUS + GAS (*H*). From week 1 to week 5, the 4 CUS groups were exposed to two stressors per day in random sequence to maximize the unpredictability. The CUS procedures were performed as described [26]. At week 6, rats were treated with saline or GAS once daily for 14 days. Following drug treatments, individual treatment groups were subdivided for behavioral testing and Western blot and Elisa analysis or BrdU immunohistochemistry: All the rats in each group were exposed to sucrose preference test, and 8 of them were then exposed to forced swim test and sacrificed for BrdU detection or Western blotting and Elisa subsequently; the remaining (*n* = 4) were exposed to Morris water maze.

To examine the direct effects on NSCs, different doses of GAS were administered to cultured NSCs for 3 or 5 days and viable cell number estimated by WST-1 assay. To investigate the protective effect of GAS against IL-1β, this cytokine (10 ng/ml) was added to the cell medium for 2 h followed by GAS treatment for 24 or 48 h. Cell number was estimated by WST-1 and BrdU assays.

2.3. Administration of GAS

GAS (purity > 99.2%) was supplied by Kunming Pharmaceutical Corporation (Kunming, China). Solubility was >300 mg/mL and bioactivity was stable for more than 2 years at room temperature in sterile water. In the present study, GAS was dissolved in saline and administered intraperitoneally to all GAS groups at the same time. The doses were chosen according to other pharmacological studies in rats [27,28] and adjusted according to our preliminary tests. In the *in vitro* study, GAS was dissolved in DMEM/F12 medium, and then diluted in DMEM/F12 to yield 10× stocks for final treatment concentrations of 5, 10, 20, and 50 μg/mL. The vehicle in the sham group was DMEM/F12 medium.

3. Behavioral testing

3.1. Sucrose preference test

The sucrose preference test (SPT) was used as indicator of anhedonia (lack of interest in rewarding stimuli), which is present in some forms of affective disorder, including depression. Specially,