Int J Clin Exp Med 2018;11(3):2361-2370
www.ijcem.com /ISSN:1940-5901/IJCEM0048527

Original Article

Astragalus polysaccharide inhibits lipopolysaccharide-induced depressive-like behaviors and inflammatory response through regulating NF-κB and MAPK signaling pathways in rats

Chengde Li[1,2*], Yu Wang[2*], Jingrong Qu[2*], Bochao Chen[2], Gong Dai[2], Xiurong Zhang[2], Ke Nie[3], Shumei Mao[2], Hongwei Sun[4]

[1]Department of TCM Pharmacology, Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China; [2]Department of Pharmacology, Key laboratory of applied Pharmacology of Shandong Province, Weifang Medical University, Weifang, Shandong, China; [3]Department of Pharmacology, School of Chinese Materia Medica, Guangdong Pharmaceutical University, Guangzhou 510006, Guangdong, China; [4]Department of Applied Psychology, Weifang Medical University, Weifang, Shandong, China. [*]Equal contributors.

Received November 10, 2016; Accepted January 28, 2018; Epub March 15, 2018; Published March 30, 2018

Abstract: Astragalus polysaccharide (APS) is a natural compound extracted from astragalus membranaceus which has an antidepressant activity. Initially APS was studied for its immunomodulatory potential, and later was found to exhibit multiple pharmacological effects, including anti-inflammatory activity. More recently APS was shown to attenuate the lipopolysaccharide (LPS) induced neuroinflammation and improve the learning and memory ability of rats. The major objectives of this study were to investigate whether APS would exhibit antidepressant effects in an animal model of depression induced by LPS, and whether this effect might be associated with regulating nuclear factor-kappaB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. Three groups of Wistar rats were injected LPS (i.p.), two groups of which were pretreated with APS (200 mg or 400 mg, i.p.). Behaviors were evaluated by forced swim test, saccharin preference test and open field test. Levels of NF-κB p65, phospho-NF-κB p65, phospho-IκBα, ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampus and hypothalamus were measured to assess the activities of NF-κB and MAPK signaling pathways. In addition, levels of TNF-α, IL-1β and IL-6 (both protein and mRNA levels) in hippocampus and hypothalamus were determined. Results showed LPS induced depressive behaviors, as well as activated the NF-κB and MAPK signaling pathways in rats. APS treatment dose-dependently alleviated depressive-like symptoms and inhibited the activation of NF-κB and MAPK signaling pathways induced by LPS. The data indicate an antidepressant-like activity of APS in a LPS-induced animal model of depression possibly via inhibition of NF-κB and MAPK signaling pathways.

Keywords: Astragalus polysaccharide, depression, inflammation, lipopolysaccharide, NF-κB, MAPK

Introduction

Major depressive disorder (MDD) is a recurrent and incapacitating mood disorder being related to high mortality and morbidity, affecting about 15% of the population worldwide [1, 2]. It is generally accepted that the pathogenesis of MDD is complex and has not been completely elucidated yet, which is far more beyond the typical "monoamine hypothesis". Based on the complexity of the pathogenesis, novel therapeutic strategies targeting at specific pathogenesis are required.

It has been proved that inflammatory mediators can interact with pathophysiologic pathways relevant to mood regulation, such as neurotransmitter metabolism, neuroendocrine function, synaptic plasticity and regional brain activity. To date, more and more studies revealed the association between the immune system activation and MDD [3-5]. Depressive patients and animal models of depression have been found to exhibit increased levels of inflammatory biomarkers in the periphery and the brain, for example TNF-α, IL-1 and IL-6 [6-8]. Furthermore, data from laboratory animals

showed that administration of IFN-α or TNF-α could induce depressive behaviors in rodents [9-11]. These data confirmed the role of inflammatory response in the pathophysiology of depression.

Regarding the signaling pathways through which the stimuli induce inflammatory response and depressive behaviors, there is a growing body of research that has begun to clarify the roles of NF-κB and MAPK signaling pathways [12, 13]. Extracellular signal-regulated kinases (ERK) 1/2 and p38 MAPK have been found to influence both the expression and the activity of the membrane reuptake pumps for both dopamine and serotonin which are neurotransmitters playing crucial roles in depression. MAPK signaling pathway has also been well demonstrated to mediate the LPS-induced neuroinflammation [14, 15] which is associated with depression. Besides, studies showed IFN-α and TNF-α induced depression was companied by p38 MAPK activation [16]. NF-κB signaling pathway is another one which promotes inflammation. Many studies have proved the activation of the NF-κB signaling pathway in depressive patients and animal models of depression [17, 18]. The activation of the NF-κB signaling pathway can lead to the overproduction of inflammatory cytokines, such as TNF-α, IL-1 and IL-6. The NF-κB activation also influences neurotransmitters, neurogenesis and the glucocorticoid receptor signaling which are involved in depression. Therefore, NF-κB is regarded as a critical mediator of stress-induced and LPS-induced depressive behaviors [18, 19]. Based on the crucial role of NF-κB and MAPK in depression, suppressing the activation of these inflammation associated signaling pathway has been tested as new therapeutical targets for depression [19-21].

Many natural compounds possessing anti-inflammatory activities have been tested to treat depression. For example, resveratrol, salidroside and EGb761 respectively attenuated the depressive-like behaviors induced LPS in rodents [22-24]; Apigenin exhibited antidepressant effect in the chronic mild stress-induced rat model of depression [25]. Herbal drug *astragalus membranaceus* has a long history of medical use for various diseases. Astragalus injection, a crude extract from *astragalus membranaceus*, has been reported to attenuate the depressive-like behaviors induced by chronic stress in rats [26]. APS, a natural compound, is one of the active pharmacological constituents of *Astragalus membranaceus*. APS has been found to have multiple pharmacological actions, including anti-inflammatory activity, and has been used to treat inflammation associated diseases such as asthma, diabetic nephropathy and liver injury in preclinical and clinical trials. Our laboratory also has tested its renoprotective, anti-inflammatory and neuroprotective effects in our previous studies [27-29]. A recent study found APS attenuated LPS-induced neuroinflammation by regulating NF-κB signaling [30]. It is also has been proved that APS could regulate the p38 MAPK signaling pathway [31, 32]. In addition, a study reported APS improved the learning and memory ability of the rats [33]. Based on the antidepressant activity of *Astragalus membranaceus* as well as the anti-inflammatory and neuroprotective effects of APS, we suppose that APS might have an antidepressant effects in inflammation induced depression. In this study, we induced depressive behaviors in rats by LPS injection and treated the rats with APS to assess its antidepressant effect. Moreover, we also investigated the effects of APS on NF-κB and MAPK signaling pathways to explore the possible mechanisms by which APS exerted its antidepressant effect in the LPS-induced depression model.

Material and methods

Animals

Wistar rats (200-220 g upon arrival) were obtained from the Laboratory Animal Center of Shandong University (Jinan, China). Animals were subjected to a 1-week acclimatization period upon the arrival and all efforts were made to minimize the number of animals used. Throughout the study, the rats were provided food and water *ad libitum* with the exception of behavioral tests. The animal room was maintained at 23-26°C on a 12-h light/dark cycle. Behavioral testing and drug injections occurred during the light phase of the cycle. All studies conformed to the requirements of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, as approved by the Committee of Animal Care and Use in our university.

APS pretreatment and LPS administration

After the acclimation period, rats were randomly assigned to control group, LPS group, APS-

200 group and APS-400 group (n=10 each). LPS (dissolved in saline at a concentration of 200 μg/ml) (Sigma Chemical Company, St Louis, MO, USA.) was intraperitoneally injected to rats in LPS group, APS-200 group and APS-400 group at a dose of 200 μg/kg, which could induce depressive-like behaviors in rats according to previous reports [34, 35]. Prior to the LPS injection, rats were pretreated with two doses of APS (APS-200 group: 200 mg/day; APS-400 group: 400 mg/day) or saline (control group and LPS group) for 5 days, and the treatments were further given for another 2 days post LPS administration.

Forced swim test

The forced swim test consisted of two exposures to a tank (46 cm tall × 20 cm in diameter) filled with water (24-26°C; 35 cm depth) in which rats could swim. The first exposure was a training phase, during which rats were placed in the water for 15 min. The test phase lasting for 5 min was carried out 24 h after the training phase. The second test phase (conducted 24 h post LPS administration) was videotaped and the immobility time in the test was scored by experienced observers that were blind to the experimental conditions. Immobility was defined as: the rat remained floating in the water without struggling and only made movement necessary to keep its head above the water.

Exploratory activity in the open-field test

Exploratory activity in the open-field test was conducted after the forced swim test. The apparatus was a wooden box (100 cm × 100 cm × 40 cm) which had 25 same squares outlined by white lines on the floor of the arena. The rats individually underwent the test. During the test, the rat was placed into the center of the arena and left free to explore the arena for 5 min and the exploratory activity of the rat was video-recorded. Rearings and crossings of the rats were scored by experienced observers that were blind to the experimental conditions. The area was wiped clean with a 75% alcohol solution after each trial.

Saccharin preference test

Rats were trained for optimal saccharin preference (0.5% solution) before testing. After 20 hr's deprivation of water, each rat was given two bottles, one containing tap water and the other with a 0.5% saccharin solution for 12 h in their individual home cages. The amount of liquid consumed from each bottle was measured and the saccharin preference was calculated as a percentage of total liquid (saccharin solution intake to saccharin solution plus water consumed).

NF-κB and MAPK signaling activity

After the behavioral tests, left hippocampus and hypothalamus were immediately removed from the brain and homogenized. The supernatant of the homogenates was collected after centrifugation at 3000 rpm for 10 min. NF-κB p65, phospho-NF-κB p65, phospho-IκBα, ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampal and hypothalamic tissue homogenate were determined using enzyme-linked immunosorbent assay (ELISA) kits, and the procedures were performed in accordance with the kit instructions (Bangyi Biotechnology Co. Ltd, Shanghai, China; Huamei Biotechnology Co. Ltd, Wuhan, China; R & D Systems, Inc., Minneapolis, MN, USA; Cell Signaling Technology, Beverly, Massachusetts, USA).

TNF-α, IL-1β and IL-6

Concentrations of inflammatory cytokines TNF-α, IL-1β and IL-6 in hippocampal and hypothalamic homogenates were assessed using radioimmunoassay kits strictly following the manufacturer's instructions (Beijing North Institute of Biological Technology Company, China).

mRNA expression of TNF-α, IL-1β and IL-6

Total tissue RNA was extracted from the hippocampus and hypothalamus samples with TRIzol reagent (TaKaRa Bio, Dalian, China), according to the manufacturer's recommended procedures. The cDNA was synthesized from 2 μg total RNA. The cDNA was amplified through quantitative real-time reverse transcription PCR analysis to determine the expression of I TNF-α, IL-1β and IL-6. The sequences of primers were: β-actin, (forward) 5'-GCAGGAGTACGATGAGTCCG-3' and (reverse) 5'-ACGCAGCTCAGTAA CAGTCC-3'; IL-1β, (forward) 5'-GCTAGGGAG CCCCC TTGTCGAG-3', and (reverse) 5'-AGG CAGGGAGGGAAACACACGTT-3'; IL-6, (forward) 5'-GTCAACTCCATCTGCCCTTCAG-3', and (reverse) 5'-GGCAGTGGC TGTCAACAACAT-3'; TNF-α, (forward) 5'-GCCACCACGCTCTTCT GTC-3', and

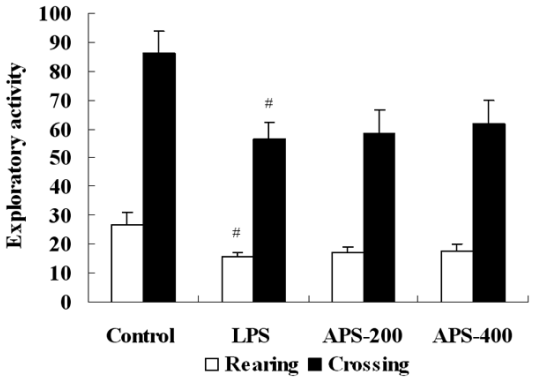

Figure 1. Effects of APS on the locomotor activity of the rats in the open field test. The locomotor activity is evaluated by the numbers of the rearings and crossings in the 5 min's test. The results are expressed as means ± SD. #*P*<0.05, versus control; *P<0.05 versus LPS, ▼*P*<0.05 versus APS-200.

(reverse) 5'-GCTACGGGCTTGTCACTCG-3'. The amplification reactions were carried out as the following: 1 min at 95°C, 35 cycles at 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 20 seconds. The mRNA expression levels of the target genes were normalized to those of β-actin.

Statistical analyses

All values were expressed as the mean ± SD. All analyses were conducted using the SPSS 13.0. The statistical significance of differences among groups was assessed with one-way ANOVA followed by the Tukey-Kramer multiple comparison. *P*<0.05 was considered to be statistically significant.

Results

APS had no significant effects on the locomotor activity of the rats

(**Figure 1**) In the open field test, rats in the LPS group showed a significant decrease in numbers of rearings and crossings when compared to the control group (respectively *P*<0.05), while APS (both 200 mg and 400 mg) treated rats displayed similar levels of rearings and crossings to the LPS group (respectively *P*>0.05).

APS reduced immobility time in forced swim test

(**Figure 2**) During the forced swim test, rats in the LPS group showed a significant increase in immobility time when compared to the control group (*P*<0.05). APS (both 200 mg and 400 mg) treated rats displayed much shorter immobility time than the LPS group (respectively *P*<0.05), and the APS-400 group displayed even shorter immobility time than the APS-200 group (*P*<0.05).

APS increased the saccharin preference

(**Figure 2**) In the saccharin preference test, rats in the LPS group showed a significant decrease in saccharin preference when compared to the control group (*P*<0.05). APS (both 200 mg and 400 mg) treated rats displayed much higher saccharin preference than the LPS group (respectively *P*<0.05), and the APS-400 group displayed higher saccharin preference than the APS-200 group (*P*<0.05).

APS inhibited the NF-*κB signaling activity*

(**Tables 1** and **2**) Rats in the LPS group had much higher levels of NF-κB p65, phospho-NF-κB p65 and phospho-IκBα in hippocampal and hypothalamic tissue homogenate than the control group (respectively *P*<0.05), indicating LPS injection activated the NF-κB signaling. APS (both 200 mg and 400 mg) treated rats displayed much lower levels of NF-κB p65, phospho-NF-κB p65 and phospho-IκBα than the LPS group (respectively *P*<0.05), and the APS-400 group had even lower levels of these parameters than the APS-200 group (respectively *P*<0.05).

APS inhibited the MAPK signaling activity

(**Tables 3** and **4**) LPS injection significantly increased levels of ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampal and hypothalamic tissue homogenate in the LPS group if compared to the control group (respectively *P*<0.05). APS significantly reversed the alterations of these parameters induced by LPS injection in the APS-200 group and the APS-400 group if compared to the LPS group (respectively *P*<0.05). Moreover, the APS-400 group displayed lower levels of these parameters than the APS-200 group (respectively *P*<0.05).

APS decreased levels of inflammatory cytokines TNF-α, IL-1β and IL-6

(**Tables 5** and **6**) Rats in the LPS group showed much higher levels of inflammatory cytokines

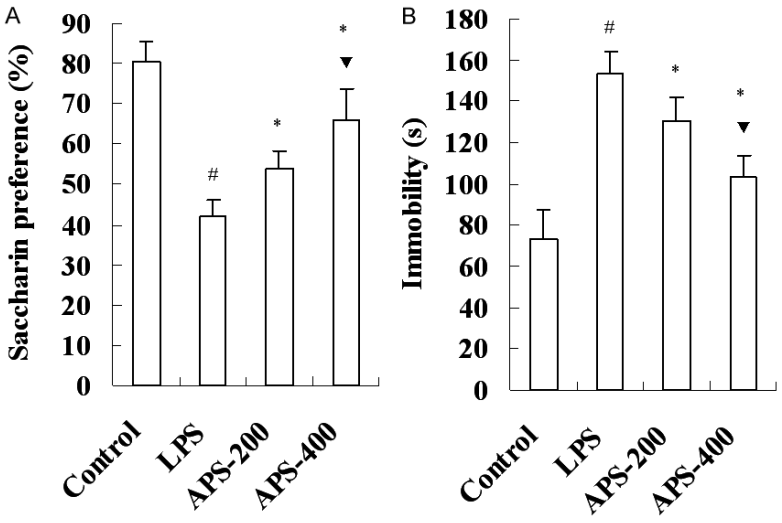

Figure 2. Effects of APS on the depressive behaviors of the rats. The depressive behaviors are evaluated by saccharin preference test and forced swim test. A: The saccharin preference was calculated as a percentage of total liquid (saccharin solution intake/saccharin solution plus water consumed). B: Immobility was defined as: the rat remained floating in the water without struggling and only made movement necessary to keep its head above the water. The immobility time in the FST was measured. The results are expressed as means ± SD. #*P*<0.05, versus control; *P<0.05 versus LPS, ▼*P*<0.05 versus APS-200.

Table 1. Effects of APS on the hippocampal NF-κB signaling activity of the rats

	NF-κB p65 (pg/ml)	phospho-NF-κB p65 (pg/ml)	phospho-IκBα (pg/ml)
Control	48.09±4.75	12.36±1.48	13.14±0.95
LPS	103.14±9.03[a]	55.26±7.01[a]	38.72±4.08[a]
APS-200	84.52±6.38[b]	40.33±5.17[b]	28.30±2.54[b]
APS-400	65.11±5.96[b,c]	26.01±3.83[b,c]	16.44±1.50[b,c]

The NF-κB signaling activity is evaluated by *measuring the levels of* NF-κB p65, phospho-NF-κB p65, phospho-IκBα in hippocampal tissue homogenate. The results are expressed as means ± SD. [a]*P*<0.05 versus control; [b]*P*<0.05 versus LPS, [c]*P*<0.05 versus APS-200.

Table 2. Effects of APS on the hypothalamic NF-κB signaling activity of the rats

	NF-κB p65 (pg/ml)	phospho-NF-κB p65 (pg/ml)	phospho-IκBα (pg/ml)
Control	42.50±4.08	11.20±1.14	12.05±1.40
LPS	78.25±6.21[a]	36.05±5.73[a]	29.14±4.01[a]
APS-200	64.70±5.63[b]	26.00±3.55[b]	22.86±2.17[b]
APS-400	53.82±5.41[b,c]	19.22±2.10[b,c]	17.19±2.05[b,c]

The NF-κB signaling activity is evaluated by *measuring the levels of* NF-κB p65, phospho-NF-κB p65, phospho-IκBα in hypothalamic tissue homogenate. The results are expressed as means ± SD. [a]*P*<0.05 versus control; [b]*P*<0.05 versus LPS, [c]*P*<0.05 versus APS-200.

TNF-α, IL-1β and IL-6 (both protein and mRNA levels) in hippocampus and hypothalamus than the control group (respectively *P*<0.05). APS (both 200 mg and 400 mg) treated rats displayed much lower levels of TNF-α, IL-1β and IL-6 (both protein and mRNA levels) than the LPS group (respectively *P*<0.05), and the APS-400 group had even lower levels of these parameters than the APS-200 group (respectively *P*<0.05).

Discussion

Astragalus membranaceus has a long history of medicinal use in traditional Chinese medicine. It has been widely used as diuretic, antiperspirant, antihypertensive, anti-inflammatory, hypoglycemic, tonic and anti-depressant treatments. Saponins, polysaccharides and flavonoids are the active pharmacological constituents of *Astragalus membranaceus*. Among the constituents, APS has been most widely studied. Initially APS was studied for its immunomodulatory potential, and later was found to exhibit multiple pharmacological effects, including anti-inflammatory activity. It has been tested to treat autoimmune diseases, diabetes, cardiovascular diseases, tumor, cell damage, and so on, and has exhibited potent therapeutical effect [27-32]. Recently, anti-inflammatory and neuroprotective effects were reported [30, 33]. However, whether it has an antidepressant activity has not been investigated. Considering the anti-inflammatory effects of APS, in the current study, we designed to investigate the effects of APS on a LPS induced animal model of depression, an inflammation associated depression model.

Table 3. Effects of APS on the hippocampal MAPK signaling activity of the rats

	ERK1/2 (pg/ml)	JNK (pg/ml)	p38 (pg/ml)	phospho-ERK1/2 (pg/ml)	phospho-JNK (pg/ml)	phospho-p38 (pg/ml)
Control	25.12±4.35	33.92±3.40	43.07±3.67	7.08±1.25	10.15±1.46	12.27±2.12
LPS	57.19±4.11[a]	69.27±5.38[a]	91.25±8.28[a]	28.37±4.88[a]	38.07±4.11[a]	46.82±6.40[a]
APS-200	45.55±3.02[b]	55.08±4.72[b]	75.22±5.07[b]	19.13±3.08[b]	24.75±3.40[b]	28.18±3.44[b]
APS-400	36.06±3.79[b,c]	46.99±4.51[b,c]	61.70±5.33[b,c]	13.01±2.10[b,c]	16.51±2.53[b,c]	19.50±2.29[b,c]

The NF-κB signaling activity is evaluated by *measuring the levels of* ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampal tissue homogenate. The results are expressed as means ± SD. [a]*P*<0.05 versus control; [b]*P*<0.05 versus LPS, [c]*P*<0.05 versus APS-200.

Table 4. Effects of APS on the hypothalamic MAPK signaling activity of the rats

	ERK1/2 (pg/ml)	JNK (pg/ml)	p38 (pg/ml)	phospho-ERK1/2 (pg/ml)	phospho-JNK (pg/ml)	phospho-p38 (pg/ml)
Control	36.09±5.63	40.95±5.85	55.09±7.38	8.89±1.33	11.70±1.88	14.47±2.11
LPS	76.01±6.40[a]	91.66±7.23[a]	124.53±10.92[a]	40.36±6.25[a]	43.01±5.53[a]	60.05±8.51[a]
APS-200	60.77±4.08[b]	73.54±6.20[b]	97.80±9.08[b]	26.17±3.54[b]	31.29±4.20[b]	41.35±5.49[b]
APS-400	48.38±5.21[b,c]	58.14±4.97[b,c]	80.72±7.11[b,c]	18.70±2.21[b,c]	22.80±3.66[b,c]	25.70±4.11[b,c]

The NF-κB signaling activity is evaluated by *measuring the levels of* ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hypothalamic tissue homogenate. The results are expressed as means ± SD. [a]*P*<0.05 versus control; [b]*P*<0.05 versus LPS, [c]*P* 0.05 versus APS-200.

Table 5. Effects of APS on the protein and mRNA expressions of inflammatory cytokines in hippocampus

	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α mRNA	IL-1β mRNA	IL-6 mRNA
Control	55.07±4.17	63.16±5.90	45.30±6.55	1.00±0.12	1.00±0.15	1.00±0.13
LPS	162.59±13.22[a]	180.15±20.05[a]	119.48±14.36[a]	2.49±0.30[a]	2.82±0.39[a]	2.27±0.19[a]
APS-200	115.28±12.99[b]	136.62±15.44[b]	88.12±12.80[b]	1.99±0.14[b]	2.04±0.18[b]	1.86±0.15[b]
APS-400	82.11±10.25[b,c]	97.27±14.31[b,c]	65.83±7.60[b,c]	1.55±0.11[b,c]	1.63±0.17[b,c]	1.50±0.11[b,c]

Levels of TNF-α, IL-1β and IL-6 in the hippocampal tissue homogenate were measured; the mRNA levels of the target genes were normalized to those of β-actin. The results are expressed as means ± SD. [a]*P*<0.05 versus control; [b]*P*<0.05 versus LPS, [c]*P*<0.05 versus APS-200.

Table 6. Effects of APS on the protein and mRNA expressions of inflammatory cytokines in hypothalamus

	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α mRNA	IL-1β mRNA	IL-6 mRNA
Control	46.87±6.55	56.00±6.21	43.92±5.87	1.00±0.09	1.00±0.12	1.00±0.14
LPS	123.05±14.36[a]	138.65±16.27[a]	101.08±14.69[a]	2.10±0.20[a]	2.76±0.29[a]	2.05±0.16[a]
APS-200	91.89±10.22[b]	101.13±13.41[b]	77.44±7.01[b]	1.70±0.11[b]	2.11±0.15[b]	1.77±0.13[b]
APS-400	65.24±8.67[b,c]	76.77±8.20[b,c]	59.10±5.89[b,c]	1.46±0.08[b,c]	1.72±0.12[b,c]	1.41±0.11[b,c]

Levels of TNF-α, IL-1β and IL-6 in the hypothalamic tissue homogenate were measured; the mRNA levels of the target genes were normalized to those of β-actin. The results are expressed as means ± SD. [a]*P*<0.05 versus control; [b]*P*<0.05 versus LPS, [c]*P*<0.05 versus APS-200.

We administrated LPS (i.p.) to the Wistar rat at dose of 200 μg/kg body weight, which is a dose that has been reported to successfully depressive-like behaviors in rats [34, 35]. Similar to the previous reports [22, 24, 36], we found LPS significantly increased the immobility time of the rats in the forced swim test which is a behavioral test widely used to evaluate the depressive state of rodents. Immobility time represents the severity of hopelessness which is a typical symptom of depression. Interestingly, rats with APS treatment (200 mg and 400 mg)

displayed much shorter immobility time than the LPS group, and the effect was dose-dependent. In addition, anhedonia, another common symptom of depression, was assessed by saccharin preference test. In line with the forced swim test, APS also dose-dependently improved the anhedonia induced by LPS. These findings indicated the dose-dependent antidepressant activity of APS in the LPS induced depression model. However, according to previous reports, LPS may elicit sickness behavior in animals which is a usual response to infection characterized by endocrine, autonomic, and behavioral changes triggered by the activation of the peripheral innate immune system [38]. As is known, sickness behavior may influence the performance of rodent animals in other behavioral tests, resulting in false results. In order to exclude the influence of sickness on other behavioral tests, sickness behavior was accessed in open field test. No differences in locomotor activity were observed between the LPS treated animals and LPS+APS treated animals. The result indicated that the attenuation of the behavioral performance in forced swim test was specific to the antidepressant effects of APS without influence from locomotor activity. All the behavioral tests confirmed the antidepressant activity of APS in LPS induced rat model of depression.

The evidence from preclinical and clinical studies supports that depression is usually accompanied by the activation of the inflammatory-response system [3-8]. And overproduction of inflammatory cytokines plays a role in the pathophysiology of depressive disorders. The cytokines has been found to influence neurogenesis and the HPA axis activity, which are both associated with depression. In preclinical studies, administration of IFN-α or TNF-α has been tested to establish animal model of depression [9-11, 16, 38]. And suppressing the over-production of these cytokines has exhibited a potent antidepressant effect [23, 39]. LPS can activate TLR-4 and induce systemic inflammation and neuroinflammation. After LPS administration, overproduction of IL-1, IFN-α and TNF-α in the peripheral tissue and brain has been found in rodents. In the current study, we analyzed the expression of IL-1β, IL-6 and TNF-α in hippocampus and hypothalamus which play a crucial role in mode regulation. Consistently with previous reports [18, 19, 40], we also found the rats in LPS group had much higher levels of inflammatory cytokines TNF-α, IL-1β and IL-6 (both protein and mRNA levels) than the control animals. Yet, APS effectively inhibited the over-expression of these cytokines (both protein and mRNA levels). Thus, the effects of APS on these cytokines may explain the positive results of APS treatment in the behavioral tests. Besides our findings, some other natural products, such as resveratrol, salidroside, EGb761, apigenin, curcumin, also exhibited anti-inflammatory and antidepressant effects in animal model of depression induced by LPS, stress and other means [22-25, 41].

Inhibition of the NF-κB and MAPK pathways has been proposed to be a major mechanism underlying the attenuation of LPS-induced inflammatory cytokine production. Activation of NF-κB signaling is involved in LPS induced inflammation response. LPS combines with TLR-4 and triggers a cascade of signaling reactions, ultimately leading to the degradation of transcription inhibition factor-κB (IκB) by phosphorylation. The phosphorylation of IκB activates NF-κB, and the activated NF-κB p65 subunit (phosphorylated NF-κB p65) is translocated to the nucleus where it promotes the transcription of target genes, such as TNF-α, IL-1β and IL-6 genes. Consistently, we observed much high levels of NF-κB p65, phospho-NF-κB p65 and phospho-IκBα in hippocampus and hypothalamus in the LPS treated rats in this study. And this was in line with the increases in levels of TNF-α, IL-1β and IL-6 (both protein and mRNA levels) LPS treated rats. In agreement with our results, other studies also demonstrated the activation of NF-κB signaling in the LPS induced animal model of depression [21, 42]. However, in this study APS (200 mg and 400 mg) inhibited the NF-κB signaling activation in a dose dependent manner, which was in line with the attenuation of inflammatory cytokine production by APS mentioned above. Similarly to our findings, there is other evidence that inhibition of NF-κB signaling contributes to the improvement of depressive-like behaviors [22, 42]. MAPK signaling, which includes JNK, ERK1/2 and p38 MAPK, is another pathway that plays an important role in inflammatory responses and is involved in pathophysiology of depression. Studies have proved that MAPK signaling is activated at least in inflammation associated depression and the managements suppressing the over-activation of the signaling manifested

antidepressant effects [16, 38]. In line with the studies that reported peripheral administration of LPS triggered neuroinflammation and activated MAPK signaling [43, 44], we found much higher levels of ERK1/2, JNK, p38 MAPK, phospho-ERK1/2, phospho-JNK and phospho-p38 MAPK in hippocampus and hypothalamus of the LPS treated rats. But rats with APS treatment displayed much lower levels of these MAPKs. All the findings suggested that APS inhibited the activation of NF-κB and MAPK signaling pathways which could mediate the inflammatory cascade elicited by LPS in rats.

Conclusions

In summary, we observed for the first time that APS exhibited antidepressant effects in an animal model of depression induced by a single administration of LPS, and the antidepressant action of APS was paralleled by significant inhibition of TNF-α, IL-1β and IL-6 expression, as well as the activity of NF-κB and MAPK signaling pathways in hippocampus and hypothalamus of the Wistar rats. The findings suggest a therapeutic potential of APS in at least inflammation associated depression. However, the potential effects of APS on the other aspects of neuroinflammation, the neurogenesis, neurotransmitter and the HPA axis activity which are associated with depression and on other types of depression models needs further study in the future work.

Acknowledgements

This work was supported by grants from National Natural Science Foundation (8137-3828), Shandong Provincial Natural Science Foundation (ZR2009CL0 45, ZR2011HL066, ZR2013CM033, ZR2015CL029), Shandong Provincial Education Department (J15LL54, J16LM11) and Administration of Traditional Chinese Medicine of Shandong Province (2013-242, 2015-237).

Disclosure of conflict of interest

None.

Address correspondence to: Ke Nie, Department of Pharmacology, School of Chinese Materia Medica, Guangdong Pharmaceutical University, Guangzhou 510006, Guangdong, China. E-mail: niekectm@sina.com; nicknk@hotmail.com; Hongwei Sun, Department of Applied Psychology, Weifang Medical University, Weifang, Shandong, China. E-mail: sincereshw@126.com; Shumei Mao, Department of Pharmacology, Key laboratory of Applied Pharmacology of Shandong Province, Weifang Medical University, Weifang, Shandong, China. E-mail: maoshumei@126.com

References

[1] Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 386: 743-800.

[2] Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS; National Comorbidity Survey Replication. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 2003; 289: 3095-3105.

[3] Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R and Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch Gen Psychiatry 2008; 65: 409-415.

[4] Licinio J, Wong ML. The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate the biological substrate of depressive symptoms, regulate stress-responsive systems, and contribute to neurotoxicity and neuroprotection. Mol Psychiatry 1999; 4: 317-327.

[5] Strawbridge R, Arnone D, Danese A, Papadopoulos A, Herane Vives A and Cleare AJ. Inflammation and clinical response to treatment in depression: a meta-analysis. Eur Neuropsychopharmacol 2015; 25: 1532-1543.

[6] Rethorst CD, Toups MS, Greer TL, Nakonezny PA, Carmody TJ, Grannemann BD, Huebinger RM, Barber RC and Trivedi MH. Pro-inflammatory cytokines as predictors of antidepressant effects of exercise in major depressive disorder. Mol Psychiatry 2013; 18: 1119-1124.

[7] O'Connor JC, Lawson MA, André C, Moreau M, Lestage J, Castanon N, Kelley KW and Dantzer R. Lipopolysaccharide-induced depressive- like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol Psychiatry 2009; 14: 511-522.

[8] Mello BS, Monte AS, McIntyre RS, Soczynska JK, Custódio CS, Cordeiro RC, Chaves JH, Vasconcelos SM, Nobre HV Jr, Florenço de Sousa FC, Hyphantis TN, Carvalho AF, Macêdo DS. Effects of doxycycline on depressive-like behavior in mice after lipopolysaccharide (LPS)

administration. J Psychiatr Res 2013; 47: 1521-1529.
[9] Kaster MP, Gadotti VM, Calixto JB, Santos AR, Rodrigues AL. Depressive-like behavior induced by tumor necrosis factor-α in mice. Neuropharmacology 2012; 62: 419-426.
[10] Fischer CW, Eskelund A, Budac DP, Tillmann S, Liebenberg N, Elfving B, Wegener G. Interferon-alpha treatment induces depression-like behaviour accompanied by elevated hippocampal quinolinic acid levels in rats. Behav Brain Res 2015; 293: 166-172.
[11] Brüning CA, Martini F, Soares SM, Savegnago L, Sampaio TB, Nogueira CW. Depressive-like behavior induced by tumor necrosis factor-α is attenuated by m-trifluoromethyl-diphenyl diselenide in mice. J Psychiatr Res 2015; 66-67: 75-83
[12] Jian CX, Li MZ, Zheng WY, He Y, Ren Y, Wu ZM, Fan QS, Hu YH, Li CJ. Tormentic acid inhibits LPS-induced inflammatory response in human gingival fibroblasts via inhibition of TLR4-mediated NF-κB and MAPK signalling pathway. Arch Oral Biol 2015; 60: 1327-1332.
[13] Wang YP, Wu Y, Li LY, Zheng J, Liu RG, Zhou JP, Yuan SY, Shang Y, Yao SL. Aspirin-triggered lipoxin A4 attenuates LPS-induced pro-inflammatory responses by inhibiting activation of NF-κB and MAPKs in BV-2 microglial cells. J Neuroinflammation 2011; 8: 95.
[14] Kim BW, Koppula S, Hong SS, Jeon SB, Kwon JH, Hwang BY, Park EJ, Choi DK. Regulation of microglia activity by glaucocalyxin-A: attenuation of lipopolysaccharide- stimulated neuroinflammation through NF-κB and p38 MAPK signaling pathways. PLoS One 2013; 8: e55792.
[15] Zeng KW, Zhang T, Fu H, Liu GX, Wang XM. Modified Wu-Zi-Yan-Zong prescription, a traditional Chinese polyherbal formula, suppresses lipopolysaccharide- induced neuroinflammatory processes in rat astrocytes via NF-κB and JNK/p38 MAPK signaling pathways. Phytomedicine 2012; 19: 122-129.
[16] Moretti M, Budni J, Freitas AE, Neis VB, Ribeiro CM, de Oliveira Balen G, Rieger DK, Leal RB, Rodrigues AL. TNF-α-induced depressive-like phenotype and p38 (MAPK) activation are abolished by ascorbic acid treatment. Eur Neuropsychopharmacol 2015; 25: 902-912.
[17] Monje FJ, Cabatic M, Divisch I, Kim EJ, Herkner KR, Binder BR, Pollak DD. Constant darkness induces IL-6-dependent depression-like behavior through the NF-κB signaling pathway. J Neurosci 2011; 31: 9075-9083.
[18] Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS. Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior. Proc Natl Acad Sci U S A 2010; 107: 2669-2674.
[19] Wang Z, Zhang Q, Yuan L, Wang S, Liu L, Yang X, Li G, Liu D. The effects of curcumin on depressive-like behavior in mice after lipopolysaccharide administration. Behav Brain Res 2014; 274: 282-290.
[20] Moretti M, Budni J, Freitas AE, Neis VB, Ribeiro CM, de Oliveira Balen G, Rieger DK, Leal RB, Rodrigues AL. TNF-α-induced depressive-like phenotype and p38(MAPK) activation are abolished by ascorbic acid treatment. Eur Neuropsychopharmacol 2015; 25: 902-912.
[21] Li R, Zhao D, Qu R, Fu Q, Ma S. The effects of apigenin on lipopolysaccharide-induced depressive-like behavior in mice. Neurosci Lett 2015; 594: 17-22.
[22] Ge L, Liu L, Liu H, Liu S, Xue H, Wang X, Yuan L, Wang Z, Liu D. Resveratrol abrogates lipopolysaccharide-induced depressive-like behavior, neuroinflammatory response, and CREB/BDNF signaling in mice. Eur J Pharmacol 2015; 768: 49-57.
[23] Zhu L, Wei T, Gao J, Chang X, He H, Miao M, Yan T. Salidroside attenuates lipopolysaccharide (LPS) induced serum cytokines and depressive-like behavior in mice. Neurosci Lett 2015; 606: 1-6.
[24] Zhao Y, Zhang Y, Pan F. The effects of EGb761 on lipopolysaccharide-induced depressive-like behaviour in C57BL/6J mice. Cent Eur J Immunol 2015; 40: 11-17.
[25] Li R, Wang X, Qin T, Qu R, Ma S. Apigenin ameliorates chronic mild stress-induced depressive behavior by inhibiting interleukin-1β production and NLRP3 inflammasome activation in the rat brain. Behav Brain Res 2015; 296: 318-325.
[26] Ding YP, Ma LM, Li YP. The effects of astragalus injection on behavior and SOD activity and MDA content of hippocampus in chronic stress depression rats. Journal of Normal University (Natural Science) 2011; 6: 75-77.
[27] Shi L, Yin F, Xin X, Mao S, Hu P, Zhao C, Sun X. Astragalus Polysaccharide Protects Astrocytes from Being Infected by HSV-1 through TLR3/NF-κB Signaling Pathway. Evid Based Complement Alternat Med 2014; 2014: 285356.
[28] Mao SM, Li CHD, Li JJ, Kang B. Effects of astragalus polysaccharides on the renal injury of diabetes rats and renal expression of aquaporin-2. Chinese Journal of Gerontology 2010; 30: 2031-2033.
[29] Li CD, Zhou WB, Sun Y, Fang L, Yu J, Mao SM. Effects of astragalus polysaccharide on Th17/Treg associated cytokines and airway inflammation in OVA-induced asthmatic rats. Chinese Journal of Gerontology 2013; 29: 1275-1278.
[30] Luo T, Qin J, Liu M, Luo J, Ding F, Wang M, Zheng L. Astragalus polysaccharide attenuates lipopolysaccharide- induced inflammatory re-

sponses in microglial cells: regulation of protein kinase B and nuclear factor-κB signaling. Inflamm Res 2015; 64: 205-212.
[31] Hai-Yan Z, Yong-Hong G, Zhi-Yao W, Bing X, Ai-Ming W, Yan-Wei X, Bei L, Li-Xia L, Li-Xin C. Astragalus polysaccharide suppresses the expression of adhesion molecules through the regulation of the p38 MAPK signaling pathway in human cardiac microvascular endothelial cells after ischemia-reperfusion injury. Evid Based Complement Alternat Med 2013; 2013: 280493.
[32] Cao Y, Ruan Y, Shen T, Huang X, Li M, Yu W, Zhu Y, Man Y, Wang S, Li J. Astragalus polysaccharide suppresses doxorubicin- induced cardiotoxicity by regulating the PI3k/Akt and p38MAPK pathways. Oxid Med Cell Longev 2014; 2014: 674219.
[33] Yao H, Gu LJ, Guo JY. Study on effects of astragalus polysaccharides in improving learning and memory functions in aged rats and its mechanism. China Journal of Chinese Materia 2014; 39: 2071-2075.
[34] Engeland CG, Kavaliers M, Ossenkopp KP. Sex differences in the effects of muramyl dipeptide and lipopolysaccharide on locomotor activity and the development of behavioral tolerance in rats. Pharmacol Biochem Behav 2003; 74: 433-447.
[35] Wang D, Lin W, Pan Y, Kuang X, Qi X, Sun H. Chronic blochade of glucocorticoid receptors by RU486 enhances lipopolysaccharide-induced depressive-like behaviour and cytokine production in rats. Brain Behav Immun 2011; 25: 706-714.
[36] Sulakhiya K, Kumar P, Jangra A, Dwivedi S, Hazarika NK, Baruah CC, Lahkar M. Honokiol abrogates lipopolysaccharide- induced depressive like behavior by impeding neuroinflammation and oxido-nitrosative stress in mice. Eur J Pharmacol 2014; 744: 124-131.
[37] Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008; 9: 46-56.
[38] Felger JC, Alagbe O, Pace TW, Woolwine BJ, Hu F, Raison CL, Miller AH. Early activation of p38 mitogen activated protein kinase is associated with interferon-alpha- induced depression and fatigue. Brain Behav Immun 2011; 25: 1094-1098.
[39] Furtado M, Katzman MA. Examining the role of neuroinflammation in major depression. Psychiatry Res 2015; 229: 27-36.
[40] Guan XT, Lin WJ, Tang MM. Comparison of stress-induced and LPS-induced depressive-like behaviors and the alterations of central proinflammatory cytokines mRNA in rats. Psych J 2015; 4: 113-122.
[41] Lopresti AL, Hood SD, Drummond PD. Multiple antidepressant potential modes of action of curcumin: a review of its anti-inflammatory, monoaminergic, antioxidant, immune-modulating and neuroprotective effects. J Psychopharmacol 2012; 26: 1512-1524.
[42] Wang Z, Zhang Q, Yuan L, Wang S, Liu L, Yang X, Li G, Liu D. The effects of curcumin on depressive-like behavior in mice after lipopolysaccharide administration. Behav Brain Res 2014; 274: 282-290.
[43] Lim JY, Hwang BY, Hwang KW, Park SY. Methylalpinumisoflavone inhibits lipopolysaccharide-induced inflammation in microglial cells by the NF-kappaB and MAPK signaling pathway. Phytother Res 2012; 26: 1948-1956.
[44] Song Y, Qu R, Zhu S, Zhang R, Ma S. Rhynchophylline attenuates LPS-induced pro-inflammatory responses through down-regulation of MAPK/NF-κB signaling pathways in primary microglia. Phytother Res 2012; 26: 1528-1533.