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Research report

Proanthocyanidin prevents lipopolysaccharide-induced depressive-like behavior in mice via neuroinflammatory pathway

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ABSTRACT

Recent studies have demonstrated neuroinflammation and increased cytokine levels are associated with depression. Aware of the efficacy the potential anti-inflammatory and antioxidative activity of proanthocyanidin, the present study was designed to investigate the effects of proanthocyanidin on lipopolysaccharide (LPS)-induced depressive-like behavior in mice. In depressive behavior tests, the immobility time of forced swimming test (FST) and tail suspension test (TST) was increased when mice were administrated a single dose of LPS (0.83 mg/kg, i.p.), whereas these alterations were reversed by proanthocyanidin treatment (80 mg/kg, p.o.). In anxiety behavior tests, all the anxiety-related parameters, such as number of buried marble, time spent in the open arm and close arm did not show statistical differences between LPS and control groups. However, anxiolytic effects were observed in marble-burying test and elevated plus maze test in single proanthocyanidin treatment and proanthocyanidin treatment together with LPS group. Further assays indicated that LPS-induced overexpression of pro-inflammatory cytokines in the hippocampus, prefrontal cortex (PFC) and amygdala were reversed by proanthocyanidin treatment. Furthermore, proanthocyanidin inhibited the LPS-induced iNOS and COX-2 overexpression, via the modulation of NF-κB in the hippocampus, PFC and amygdala. Taken together, proanthocyanidin may be an effective therapeutic agent for LPS-induced depressive-like behaviors via its potent anti-inflammatory property.

1. Introduction

For years, accumulating evidence reveals that neuroinflammation plays an important role in the pathophysiology of depression (Miller and Raison, 2015; Krogh et al., 2014). Recently, studies found that neuroinflammation in patients with depression were characterized by the release of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and nuclear factor-kappaB (NF-κB) activation (Monje et al., 2011; Koo et al., 2010). Similar results had been demonstrated in some animal models as well. These animals exhibited depressive like behavior, with high expression of inflammatory mediators and increased nitric oxide synthase (iNOS) level in the prefrontal cortex (PFC) and hippocampus (Janssen et al., 2010; Hannestad et al., 2011; Chen et al., 2016). Therefore, prevention of inflammatory disturbances has been acknowledged as a potential avenue for treatment of depression.

As the lipoglycans and endotoxins, Lipopolysaccharides (LPS) induces a strong response from normal animal immune systems. The acute administration of cytokine inducer LPS is a widely-accepted animal model to investigate the relationship between neuroinflammation and depressive symptoms (Mello et al., 2013; Wang et al., 2014; Xi et al., 2016). Furthermore, some studies revealed that LPS-treated mice exhibited abnormal expression of pro-inflammatory cytokines and increased oxidative damage (Molteni et al., 2013; Tomaz et al., 2014; Zhu et al., 2015; Sulakhiya et al., 2016). The depressive-like behaviors induced by administration of LPS, such as hypoactivity in the force swimming test and the tail suspension test, can be reversed by antidepressant treatments (Yirmiya, 1996; Ohgi et al., 2013). In this study, we chose single LPS administration as animal model of depression to evaluate acute therapeutic effects of drugs on inflammatory induced depression. If the drug has the acute therapeutic effect, chronic LPS injection will be used for assessing long-term therapeutic effect of drugs in further study.

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Grape (Vitis vinifera) is one of the most widely consumed fruits worldwide and grape seed extract consists of ~90% proanthocyanidins and 7% other polyphenols (flavonoids) (Peng et al., 2005). Proanthocyanidin is a kind of phenolic product (oligonols are catechin-type monomers, dimmers and trimers, as well as oligomeric proanthocyanidins) present in plant. Experimental and clinical studies have shown that proanthocyanidin has a variety of pharmacological effects, including potent anti-inflammatory and antioxidant effect (Preuss et al., 2000; Uchida et al., 2008; Sato et al., 2001). Recently, a growing number of studies have found that the proanthocyanidin in grape seed extract possess strong anti-inflammatory activity and would be a potential therapeutic agent for some neuroinflammatory diseases, such as mood disorders and Alzheimer's disease (Aruoma et al., 2006; Mazzio et al., 1998; Xu et al., 2005).

In the present study, we aimed to investigate the ability of proanthocyanidin to modulate depressive like and anxiety like behaviors of LPS-treated mice. In addition, we measured the expressions of NF-κB, pro-inflammatory cytokines, iNOs and TNFα in the three important brain regions, PFC, amygdala and hippocampus, which are implicated in important behavioral functions, such as emotion, motivation, learning and memory (Butterweck et al., 2002; Rosen et al., 2015) to further confirm the participation of neuroinflammatory in proanthocyanidin treatment.

2. Materials and methods

2.1. Animals

Six-week-old male ICR (Institute of Cancer Research) mice (20–22 g) were obtained from the Shanghai Animal Center, Chinese Academy of Sciences. Upon arrival, the mice were group-housed four per cage and acclimatized to a colony room with controlled ambient temperature (22 ± 1 °C), humidity (50 ± 10%) and a light/dark cycle (12:12 h, lights on 7:00 AM). All experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and approved by the Wenzhou Medical College Committee on Animal Care and Use.

2.2. Experimental protocols

Proanthocyanidin (95%) was purchased from Tianjin Jianfeng Natural Product R & D Co., Ltd (Tianjin, China), which contained catechin-type monomers and oligomeric proanthocyanidin (60–80% oligomers), the easily absorbed forms. Proanthocyanidin was dissolved in 0.5% sodium carboxymethylcellulose (CMC-Na). For acute inflammation experiment, mice were received vehicle (CMC-Na) or proanthocyanidin (20, 40, 80 mg/kg, p.o.) for 7 consecutive days before LPS injection. LPS (Sigma, catalogue number:L2880) was dissolved in sterile saline, mice injected with saline or LPS (0.83 mg/kg) after 1 h proanthocyanidin administration on the 7th day (Fig. 1). The dose of LPS used was selected based on previous studies (O'Connor et al., 2009; Sulakhiya et al., 2016). Mice were randomly segregated into six groups: control, control + proanthocyanidin (dose in 80 mg/kg), LPS-treated, LPS + proanthocyanidin (dose in 20, 40, 80 mg/kg). Each group contains 2 subgroup (n = 10 in each subgroup), one is used for evaluating depressive behavior, the other is for anxiety behavior test. The behavior tests were assessed 24 h following injection of saline or LPS. All animals (n = 120) received locomotor activity test before depressive or anxiety behavioral tests. Furthermore, the effect of single acute proanthocyanidin administration on locomotor activity in LPS and control mice were tested in another independent experiment.

2.3. Locomotor activity

The assessment of locomotor activity was carried out as previously described (Yu et al., 2016; Xi et al., 2016). Mice was placed in a square chamber which was connected to photoelectric cells with light beams passing through the chamber for 15 min. During this period, number of light beam breaks was recorded. Mice were performed a training session for 5 min (pre-test), and then locomotion counts were recorded during the 10 min testing period. Mice were submitted to locomotor activity test 3 h, 6 h and 24 h after LPS injection.

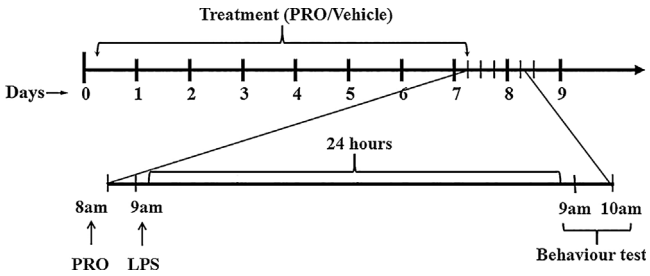

Fig 1. Experimental protocol schedule. Mice received administration of proanthocyanidin (20, 40, 80 mg/kg, p.o.) or vehicle (carboxymethylcellulose sodium, CMC-Na, p.o.) for 7 days. LPS or vehicle were injected on day 7 after proanthocyanidin treatment, then behavior tests were done at 24 h after LPS injection. Animals were sacrificed immediately after behavior test for neurochemical analysis. PRO: proanthocyanidin.

2.4. Forced swimming test

Forced swimming test (FST) has been used to identify depressive like behavior in animals (Porsolt et al., 1977, 1978). Mice (n = 10/group, different from those used for TST) were submitted to FST after LPS injection at 24 h. Briefly, mice firstly underwent a swimming-stress session for 15 min (pre-test) in a glass cylinder (height: 25 cm; diameter: 10 cm; containing 10 cm of water at 24 ± 1 °C). After 24 h, mice were placed into cylinder again for 6 min (test session). The duration that animals remained immobile during a 6-min observation period was recorded. The last 4 min of the 6-min test was scored for the immobility time. Mice was judged to be immobile when it ceased struggling and remained floating motionless in the water, or made only small movements necessary to keep its head above water.

2.5. Tail suspension test

The tail suspension test (TST) was based on the method of Steru (Steru et al., 1985) as our previous study (Yu et al., 2016). Mice (n = 10/group, different from those used for FST) were submitted to TST 24 h after LPS administration. Animals were suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the tip of the tail. The time during which mice remained immobile was quantified during the test period of 6 min.

2.6. Marble-burying test

Marble-burying test was carried out as previously described (Yu et al., 2015). In brief, mice (n = 10/group, different from those used for depressive behavior tests) were placed individually in a polypropylene cage containing 9 clean glass marbles evenly spaced on 5 cm deep sawdust. Ten minutes later, mice were removed, and the number of marbles at least one-half buried in the sawdust was recorded. Mice were submitted to marble-burying test 24 h after LPS injection.

2.7. Elevated plus maze test

The elevated plus maze test was carried out as previously described (Luo et al., 2014). In brief, animals (n = 10/group, different from those used for depressive behavior tests) were placed on the apparatus of an elevated platform, which consists of two open arms, two closed arms and a central platform. In the beginning of the test, mice were located