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Gypenosides attenuate lipopolysaccharide-induced optic neuritis in rats

Fang Wang[a], Yalong Dang[b], Jing Wang[a], Ting Zhou[a], Yu Zhu[a,*]

[a] Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450001, People's Republic of China
[b] Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA 15217, United States

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ABSTRACT

Purpose: To evaluate the effect of gypenosides (GPs) on lipopolysaccharide (LPS)-induced optic neuritis rats.
Methods: Optic neuritis was induced by a single microinjection of LPS into the optic nerve of Sprague Dawley rats. GPs (400 mg/kg) was administrated by gavage for 21 days. The optic nerve structure changes and demyelination were observed after hematoxylin & eosin and Luxol-fast blue staining. Apoptosis of retinal ganglion cells (RGCs) was evaluated using Brn3a-TUNEL double staining. Expression of CD68 and glial fibrillary acidic protein (GFAP) were detected using immunofluorescence staining. The mRNA levels of inflammatory factors were measured using quantitative real-time PCR. The protein expression levels in the signal transducer and activator of transcription (STAT) and nuclear factor-κB (NF-κB) pathways were detected using Western blot.
Results: GPs treatment prevented the optic nerve structure changes and demyelination in the rats with optic neuritis. GPs treatment downregulated LPS-induced overexpressions of CD68, GFAP and pro-inflammatory factors. GPs treatment inhibited STAT1 and 3 phosphorylation and NF-κB nuclear translocation in the optic nerve and retina of rats with optic neuritis.
Conclusion: GPs attenuate LPS-induced inflammation, demyelination and optic nerve damage which may be associated with the inhibition of the NF-κB and STAT pathways.

1. Introduction

Optic neuritis is an inflammatory disease in the optic nerve, which is composed by fibers derived from retinal ganglion cells (RGCs), different types of glial cells, and surrounded by meninges. Optic neuritis can lead to vision loss, dyschromatopsia, and orbital pain (Toosy et al., 2014), and more commonly attacks women (Jin et al., 1998). With an annual incidence about 5 in 100,000, optic neuritis is believed to be the most common optic neuropathy in young adults (Martinez-Lapiscina et al., 2014). Acute optic neuritis can also occur independently as idiopathic or primary seizure (Hickman et al., 2002). Optic neuritis can be caused by various conditions, including multiple sclerosis, infectious diseases, and autoimmune disorders (Frigui et al., 2011; Horwitz et al., 2014; Kallenbach and Frederiksen, 2008). In multiple sclerosis-associated and idiopathic optic neuritis, the inflammatory autoimmune reaction is considered to be the cause of optic nerve demyelination. Patients with optic neuritis suffer from a severe acute vision damage for 1 week and will be improved within 2–8 weeks. However, some of the patients may still have residual visual dysfunction. Moreover, recurrence of optic neuritis may lead to RGC loss and optic nerve atrophy (Trip et al., 2005; You et al., 2013), which may cause permanent visual deficit which varies from small scotoma to complete blindness. In addition, optic neuritis is associated with demyelinating diseases of the central nervous system (CNS) such as multiple sclerosis and neuromyelitis optica (Avasarala, 2015; Kale, 2016; Optic Neuritis Study, 2008).

Currently, corticosteroids are the primary medications available for optic neuritis. corticosteroids treatment is clinically effective in speeding short-term recovery in optic neuritis patients, but not in long-term visual improvement (Beck and Gal, 2008; Brusaferri and Candelise, 2000). Corticosteroids also do not prevent optic nerve atrophy (Hickman et al., 2003) and their effect on RGC in optic neuritis is controversial (Diem et al., 2003; Dimitriu et al., 2008; Dutt et al., 2010). Thus, given the importance of axonal and RGC damage in the pathological feature of optic neuritis, drugs with both anti-inflammatory and neuroprotective properties may be beneficial for optic neuritis and need to be developed.

Gypenosides (GPs) are a kind of saponin isolated from *Gynostemma pentaphyllum*. Studies have demonstrated various bioactivities of GPs including myocardial protection (Yu et al., 2016a), anti-inflammation (Cai et al., 2016; Wan and Zhao, 2017), anti-fibrosis (Song et al., 2017), and anti-cancer (Liao et al., 2016). In addition, GPs have neuroprotective effects on various CNS diseases (Mu et al., 2016; Wang et al., 2010; Wang et al., 2014; Zhang et al., 2011). As reported, GPs could protect retinal nerve fibers and axons in a myelin oligodendrocyte

* Corresponding author at: Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, East Jianshe Road, Zhengzhou 450001, People's Republic of China.
E-mail address: 13673666718@163.com (Y. Zhu).

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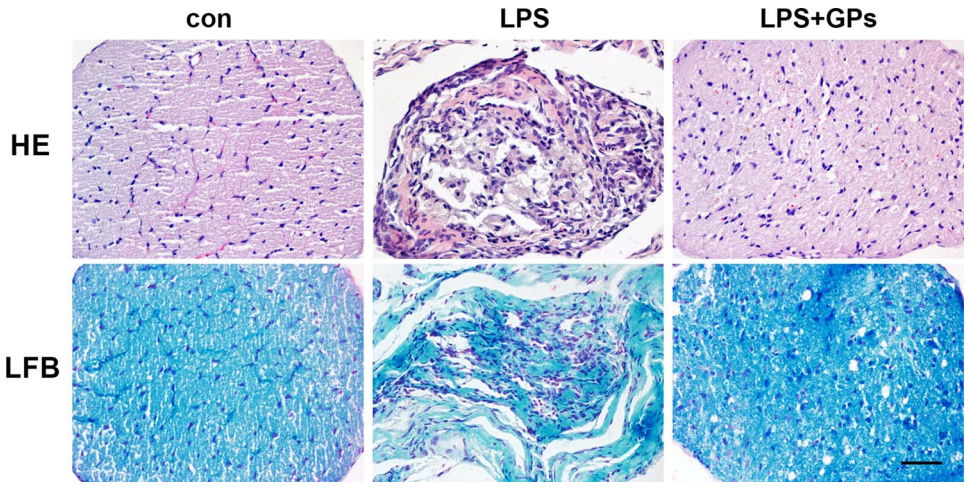

Fig. 1. GPs attenuated optic nerve structure changes (by H&E staining, upper panel) and demyelination (by LFB staining, lower panel) in rats with optic neuritis. GPs attenuated LPS-induced obvious optic nerve injury and demyelination. Scale bar: 50 μm.

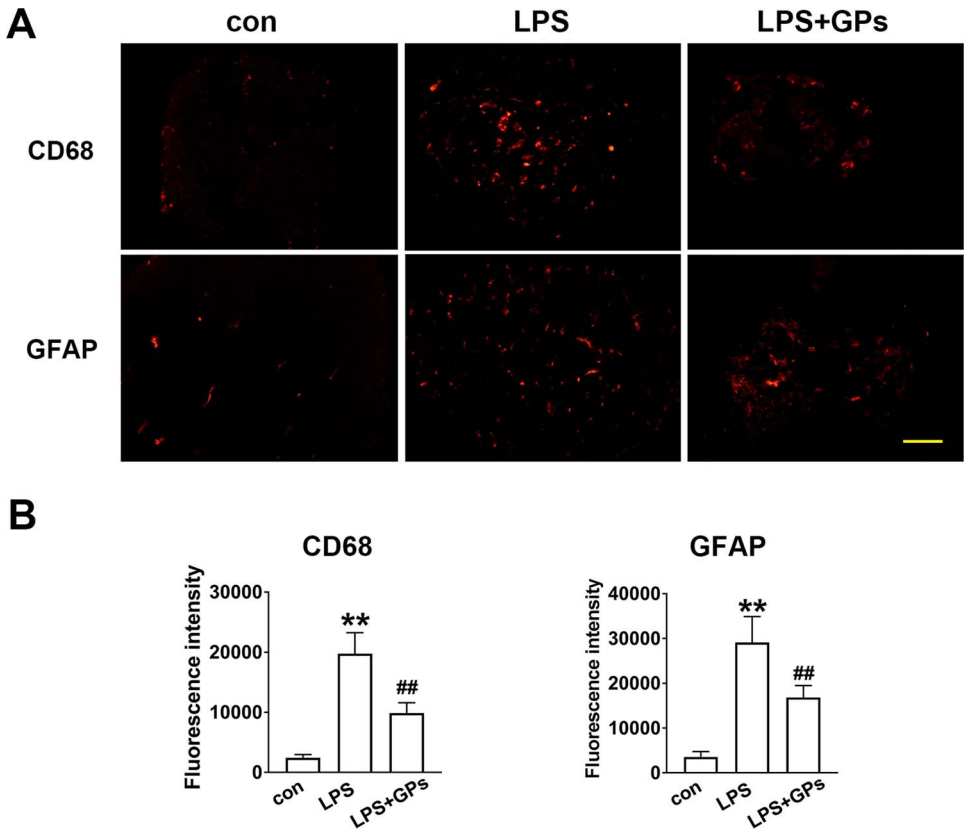

Fig. 2. GPs inhibited the LPS-induced activation of microglia/macrophages and astrocytes in optic nerve. (A) Immunofluorescence staining of CD68 and GFAP in optic nerve. Scale bar: 50 μm. (B) Immunofluorescence intensity of $CD68^+$ and $GFAP^+$ cells. Data were presented as mean ± SD, n = 6. $^{**}P < 0.01$ versus control group, $^{\#\#}P < 0.01$ versus LPS group.

glycoprotein (MOG)-induced autoimmune optic neuritis rat model (Zhang et al., 2017). In the present study, a lipopolysaccharide (LPS)-induced optic neuritis rat model, which can mimic the retina and optic nerve damages in optic neuritis without systematic inflammation (Aranda et al., 2015, Aranda et al., 2016), was used to evaluate the effects of GPs on inflammation and optic nerve damage and investigate the associated molecular signaling pathway.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (180–200 g, 8–10 weeks old) were obtained from the Laboratory Animal Center of Henan Province (Zhengzhou, China). All the animals were kept in a standard animal room under a 12-h light/dark cycle and 22 ± 2 °C with food and water available ad libitum. All protocols were approved by the institutional committees of the Animal Research Committee and Animal Ethics Committee of Zhengzhou University.