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Anti-inflammatory effects of anisalcohol on lipopolysaccharide-stimulated BV2 microglia via selective modulation of microglia polarization and down-regulation of NF-κB p65 and JNK activation

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ABSTRACT

Inflammation plays a pivotal role in the pathogenesis of ischemic stroke. The inhibition of inflammation appears to be a potential therapeutic strategy for neuro-inflammatory injury after ischemic stroke. In response to cerebral ischemia, resident microglia and infiltrated macrophages from the damaged blood–brain barrier are activated. Microglia activation appears to be a double-edged sword. Activated microglia migrate to the damaged neuron, change their phenotype to M1 or M2, and become involved in nerve damage and repair. M1 phenotype microglia express multiple inflammatory factors to exacerbate secondary brain injury, while those of M2 phenotype release anti-inflammatory factors to promote brain recovery after ischemic stroke. Therefore, the regulation of microglia M1/M2 phenotype after ischemic stroke is crucial for brain repair. The present study aimed to investigate the anti-inflammatory effect of anisalcohol (*p*-methoxybenzyl alcohol, PMBA), a phenolic compound from *Gastrodia elata* Blume, which has been shown to reduce cerebral ischemic injury in rodents. However, no studies have specifically addressed whether PMBA can selectively modulate microglia polarization. In this study, lipopolysaccharide-stimulated BV2 microglia were used to assess the anti-inflammatory effect of PMBA. The results revealed that PMBA significantly reduced the lipopolysaccharide-induced production of tumour necrosis factor α, prostaglandin E_2, and nitric oxide, without causing cell toxicity. In addition, it increased anti-inflammatory interleukin-10 and transforming growth factor-β. Phenotypic analysis of LPS-stimulated BV2 microglia showed that PMBA significantly down-regulated the expression of the M1 marker CD16/32 and up-regulated that of the M2 marker CD206. Moreover, PMBA suppressed NF-κB activation and inhibited the phosphorylation of JNK in LPS-stimulated BV2 microglia. Collectively, our data demonstrate that PMBA can inhibit M1 transformation and promote M2 transformation of microglia, thus attenuating the production of inflammatory mediators and cytokines. The modulation of microglia M1/M2 polarization may involve multiple mechanisms, mainly, the inhibition of NF-κB and MAPK activation. These findings suggest that PMBA acts as an anti-inflammatory factor and is a possible therapeutic candidate for diseases such as ischemic stroke, where inflammation is a central hallmark.

1. Introduction

Ischemic stroke is one of the most common and leading causes of death and permanent disability worldwide (Go et al., 2014; Liu et al., 2011; VanGilder et al., 2012). The rupture of carotid atherosclerotic plaques leads to the development of cerebrovascular thrombosis (Kong et al., 2016). If a brain artery is completely occluded, brain tissue supplied by that artery will become ischemic and hypoxic (Gronberg et al., 2013). Thrombolytic therapy with tissue-type plasminogen activator (t-PA) administered within 4.5 h of symptom onset is the only therapy supported by randomized clinical trials to reduce long-term disability in acute ischemic stroke (Lyerly et al., 2014). However, the outcome is strongly dependent on the timing of the treatment, and few patients benefit from accepting thrombolytic treatment due to the narrow thrombolytic therapeutic time window after stroke. Medication beyond the time window may cause cerebral ischemia reperfusion injury (CIRI), which can trigger a cascade of deleterious cellular and molecular events (Denes et al., 2011). Thus, reducing CIRI is thought to be an effective strategy for the treatment of ischemic stroke. Although the mechanisms of CIRI are complex and involve the interaction of numerous pathophysiological processes, there is accumulating evidence that the inflammation response occurs throughout the pathological

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course of CIRI (Cheng and Lee, 2016; Guo et al., 2012; Hughes et al., 2012; Wu et al., 2017; Yuan et al., 2014). In the ischemic stroke environment, resident microglia function is the first and main form of active immune defence in the central nervous system (Gaire et al., 2015; Lourbopoulos et al., 2015; Morrison and Filosa, 2013). In the acute stage, microglia are activated in response to damaged neuron-derived pro-inflammatory mediators (Biber et al., 2001; Smith et al., 2012). The activated microglia can adopt two phenotypes and play a dual role in ischemic stroke (Dudvarski Stankovic et al., 2016; Patel et al., 2013; Perego et al., 2011). Within 24 h of symptom onset, microglia show a transient M2 phenotype in the ischemic penumbra and express high levels of transforming growth factor beta (TGF-β), interleukin-10 (IL-10), and insulin-like growth factor-1 (IGF-1) to prolong neuron survival and minimize cerebral infarct volume (Bell-Temin et al., 2015). Later, activated microglia shift to the M1 phenotype, characterized by the expression of multiple pro-inflammatory factors, such as tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), which induce brain damage (Pan et al., 2015). In sub-acute stages of cerebral infarction, sustained neuro-inflammation induced by over-activated microglia damages neuron and cerebrovascular endothelial cells, which incites tissue destruction and worsens functional outcome. For this reason, microglia are thought to be a key target for therapeutic intervention against ischemic stroke (Espinosa-Garcia et al., 2017; Igarashi et al., 2003; Kato et al., 2003; Park et al., 2012; Wang et al., 2016). Inhibiting the M1 phenotype or promoting polarization toward the M2 phenotype has been suggested as an effective therapeutic strategy in ischemic stroke (Crain et al., 2013; Hu et al., 2012; Lee et al., 2016; Pan et al., 2015; Perego et al., 2016).

Anisalcohol (*p*-methoxybenzyl alcohol, PMBA, Fig. 1), a phenolic compound widely used for food flavouring and as a pharmaceutical intermediate, was originally isolated from *Gastrodia elata* Blume (GEB) (Duan et al., 2013). Our previous studies revealed an important protective effect of GEB on cerebral ischemia-reperfusion injury (Dai et al., 2017; Duan et al., 2015). PMBA may be the bioactive compound in this neuroprotective effect. However, it is unclear whether PMBA has an effect on the polarization of microglia. In this study, we investigated the anti-inflammatory effect of PMBA in lipopolysaccharide (LPS)-activated microglia and its possible mechanisms.

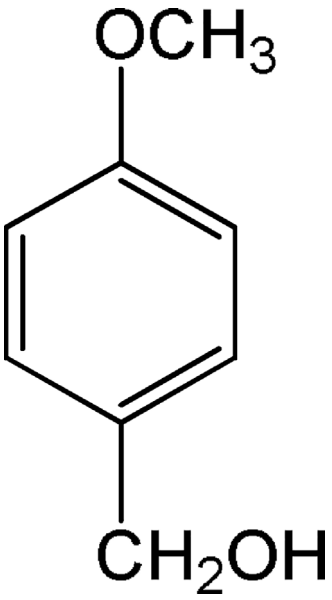

Fig. 1. Chemical structure of anisalcohol.

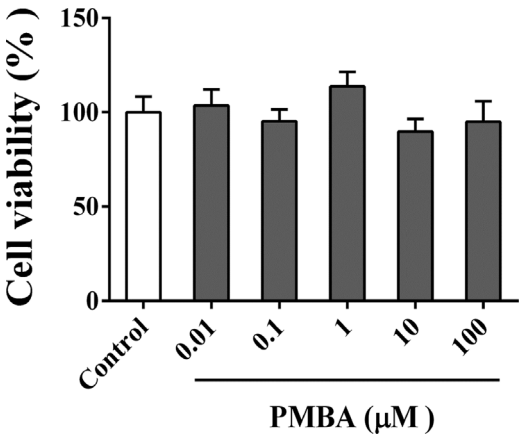

Fig. 2. Effects of PMBA on BV2 microglial cell viability. Cells were treated with various concentrations of PMBA for 24 h. Cell viability was determined by measuring the absorbance at 490 nm after addition of the MTS reagent, and the results are expressed as the percentage of surviving cells over control cells (without PMBA treatment). The values shown are the mean ± SEM from three independent experiments, each performed in triplicate.

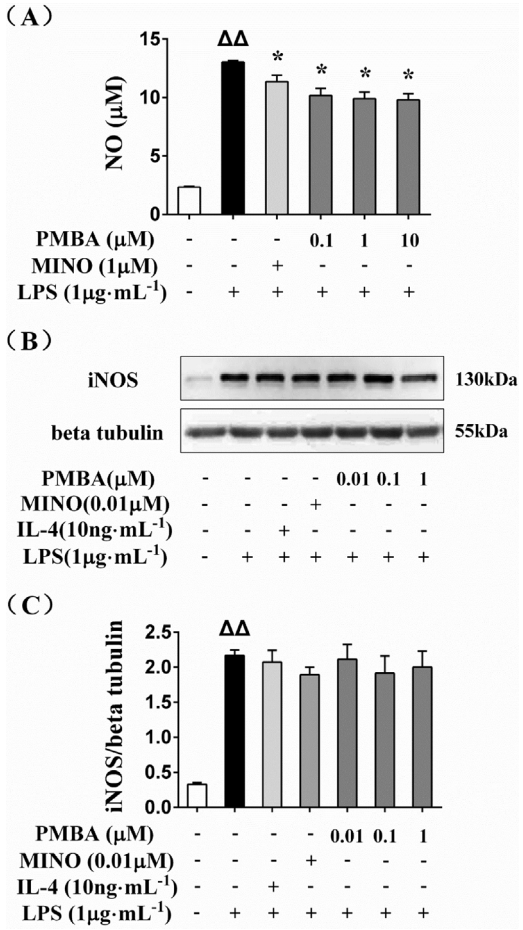

Fig. 3. Effect of PMBA on LPS-induced NO production and iNOS protein expression. BV2 microglial cells were pre-treated with various concentrations of PMBA for 24 h and then incubated with LPS (1 μg/mL) for 24 h. (A) Nitrite content was measured using the Griess reaction. (B, C) iNOS protein expression was measured by western blotting. $^{\Delta\Delta}P < 0.01$ vs. the sham group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. LPS-treated group.