Histol Histopathol (2020) 35: 1229-1250

**http://www.hh.um.es**

Histology and Histopathology
*From Cell Biology to Tissue Engineering*

## *Review*

# Microglia mediated neuroinflammation - signaling regulation and therapeutic considerations with special reference to some natural compounds

**Yue-yi Yao[1], Eng-Ang Ling[2] and Di Lu[1]**

[1]Technology Transfer Center, Kunming Medical University, Kunming, China and [2]Department of Anatomy, Young Loo Lin School of Medicine, National University of Singapore, Singapore

**Summary.** Neuroinflammation plays a central role in multiple neurodegenerative diseases and neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), cerebral ischemic injury etc. In this connection, microglia, the key players in the central nervous system, mediate the inflammatory response process. In brain injuries, activated microglia can clear the cellular debris and invading pathogens and release neurotrophic factors; however, prolonged microglia activation may cause neuronal death through excessive release of inflammatory mediators. Therefore, it is of paramount importance to understand the underlying molecular mechanisms of microglia activation to design an effective therapeutic strategy to alleviate neuronal injury. Recent studies have shown that some natural compounds and herbal extracts possess anti-inflammatory properties that may suppress microglial activation and ameliorate neuroinflammation and hence are neuroprotective. In this review, we will update some of the common signaling pathways that regulate microglia activation. Among the various signaling pathways, the Notch-1, mitogen-activated protein kinases (MAPKs), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ϰB) have been reported to exacerbate microglia mediated neuroinflammation that is implicated in different neuropathological diseases. The search for natural compounds or agents, specifically those derived from natural herbal extracts such as Gastrodin, scutellarin, Rg1 etc. has been the focus of many of our recent studies because they have been found to regulate microglia activation. The pharmacological effects of these agents and their potential mechanisms for regulating microglia activation are systematically reviewed here for a fuller understanding of their biochemical action and therapeutic potential for treatment of microglia mediated neuropathological diseases.

**Key words:** Neuroinflammation, Activated microglia, Signaling pathways, Natural compounds, Anti-inflammatory effects

*Offprint requests to:* Professor Di Lu, Technology Transfer Center, Kunming Medical University, 1168 West Chunrong Road, Yuhua Avenue, Chenggong District, Kunming 650500, Yunnan Province, P.R. China. e-mail: ludi20040609@126.com or Emeritus Professor Eng-Ang Ling, Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597. e-mail: antlea@nus.edu.sg

DOI: 10.14670/HH-18-239

**Abbreviations.** ACE, angiotensin converting enzyme; AD, Alzheimer's disease; AngII, angiotensin II; AT1, angiotensin II type 1 receptor; AT2, angiotensin II type 2 receptor; Aβ, β-amyloid; BBB, blood-brain barrier; CNS, central nervous system; COX-2, cyclooxygenase-2; CREB, cAMP responsive element binding; ERK, extracellular signal-regulated kinase; GPER, G protein-coupled estrogen receptor 1; GSK, glycogen synthase kinase; I/R, ischemia and reperfusion; IKK, IκB kinase complex; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; MCAO, middle cerebral artery occlusion; MKK, MAPK kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NICD, notch intracellular domain; NO, nitric oxide; PD, Parkinson's disease; PLC, phospholipase C; PTEN, Phosphatase and tensin homolog; RAS, Renin-angiotensin systems; SIRT3, sirtuin 3; TNF-α, tumor necrosis factor-alpha; TRAF6, TNF receptor-associated factor 6.

## Introduction

It is well documented that microglia play an important role in the immune reaction to harmful stimulation in the CNS. The main function of activated microglia in the neuroinflammatory response is to remove necrotic cells and tissues caused by pathogenic infections, disease and other damages. Uncontrolled microglia mediated neuroinflammation, however, contributes to the progress of neurodegenerative diseases including PD, AD, Huntington's disease (Jacobs and Tavitian, 2012) etc.

Microglia residing in the CNS are the primary innate immune cells and they play a critical role in maintaining the homeostasis of the brain (Venneti et al., 2009). They are involved in the immunity, neurogenesis, synaptogenesis, neurotrophic support, phagocytosis of cellular debris and maintaining CNS integrity and homeostasis. Early understanding of microglia was that they acted as tissue-resident macrophages or peripheral myeloid cells in the CNS. Accumulating evidence in recent years has led us to a better understanding of the roles of microglia in CNS both in physiological and pathological conditions. The current concept holds the view that microglia arise from the yolk sac-derived primitive macrophages during embryogenesis (Alliot et al., 1999; Ginhoux et al., 2010). Microglia exist in two different phenotypes, the so-called resting state where they appear as ramified cells, and the activated state where they often assume an amoeboidic/round form. Upon activation, microglia release an array of proinflammatory mediators such as chemokines, cytokines, inducible nitric oxide synthase/nitric oxide (iNOS/NO) and cyclooxygenase-2 (COX-2) that may lead to neuroinflammation detrimental to the brain tissue (Dheen et al., 2007; Lull and Block, 2010).

Injuries, β-amyloid (Aβ), lipopolysaccharide (LPS) and lipoteichoic acids are the causes of microglia activation and induce neuroinflammatory response. This would elicit activation and/or production of various signaling pathways and proinflammatory mediators including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), prostaglandin E2, IL-6, reactive oxygen species (ROS), reactive nitrogen species (RNS), COX-2, MAPKs, NF-ϰB, matrix metalloproteases, peroxisome, proliferation activated receptor-gamma, monocyte chemoattractant protein 1 (MCP-1) and c-Jun N-terminal kinases (JNK) (Lull and Block, 2010; Parkhurst and Gan, 2010; Greter and Merad, 2013). Since inflammatory responses in the CNS can lead to neuronal damage and subsequently neurodegeneration diseases, it is important to attenuate the production of proinflammatory and inflammatory mediators (Du et al., 2017). Regulating microglia activation therefore would be a potential therapeutic strategy to mitigate the process of inflammatory responses in the CNS. In order to better understand microglia plasticity and homeostatic regulation, many studies have been carried out to clarify the signaling network/mechanisms that drive microglial activation, especially under pathological conditions.

Recent studies have aimed at identifying agents that may regulate the activation of microglial cells, with the ultimate aim of preventing or treating brain diseases associated with chronic inflammation. Some of these studies have involved the use of natural herbal compounds for attenuation of microglia activation as discussed presently. Thus, in the search for candidate agents, notably those derived from herbal and natural products that are endowed with anti-inflammatory properties, several natural agents have been identified and evaluated. For example, the active ingredients from some Chinese herbal compounds and natural products with anti-inflammatory and neuroprotective effects have been explored in depth (Li et al., 2014; Yuan et al., 2015; Luo et al., 2017). Here we will first review the possible molecular mechanism and some signaling pathways involved in microglia activation as elicited in various pathological conditions. Next, we will discuss the use of natural products which have been reported to quell the inflammatory response mediated by microglia.

## Signaling pathways and epigenetic mechanisms that regulate microglia activation

### *Notch signaling*

In the CNS, Notch signaling pathway is one of the most conserved pathways known to regulate neural development. There is compelling evidence indicating that Notch signaling not only can it influence the developmental processes in postnatal life but is also involved in CNS pathogenesis. It is unequivocal that the Notch signaling pathway can modulate the function of microglia in neuroinflammatory diseases (Arumugam et al., 2006). The Notch signaling pathway is composed of Notch ligands (Delta-like 1, 3, 4, Jagged1, Jagged2), Notch receptors (Notch1-4), DNA binding protein recombining binding protein suppressor of hairless and the transcription factor hairy and enhancer of split. The Notch signaling pathway is activated by interaction of Notch receptor and its ligand between two adjacent cells. After this, the intracellular domain of notch receptor is hydrolyzed by γ-secretase, whereby the Notch intracellular domain (NICD) is released. NICD is then translocated into the nucleus and binds to the transcriptional repressor recombining binding protein suppressor of hairless, thereby activating the transcription of target genes such as hairy and enhancer of split (HES, HEY, and HERP) (Bray, 2006). It has been reported that (Sun et al., 2012b) the Notch signaling pathway is significantly activated after nerve injury. Very interestingly, in Notch antisense mice and in normal mice treated with γ-secretase inhibitors that block the proteolytic cleavage and activation of Notch, the brain damage and post-ischemic inflammation were significantly attenuated (Wei et al., 2011). Moreover, activation of the Notch signaling pathway promoted microglia activation in a rat model of temporal lobe

epilepsy and focal cerebral ischemia (Cao et al., 2010; Wei et al., 2011; Wu et al., 2018). Notch expression was upregulated in activated microglia in cerebral ischemia, while Notch-1 antisense mice exhibited significantly decreased numbers of activated microglia and reduced proinflammatory cytokine expression in the ipsilateral ischemic cortices compared to nontransgenic mice (Yao et al., 2013a). Additionally, it has been reported that hypoxia can induce the activation of Notch-1 signaling in primary microglia and BV-2 cells (Yao et al., 2013b; Yuan et al., 2015). It was found that blocking Notch-1 suppressed protein expression of IL-1β, TNF-α, monocyte chemoattractant protein 1 (MCP-1) and iNOS in BV-2 cells. In microglial cells pretreated with N-(N-(3,5-difluorophenacetyl)-lalany1)-S-phenyglycinet-butylester, the γ-secretase inhibitor, followed by LPS stimulation, the expression of the hairy and enhancer of split-1 (Hes-1) along with TNF-α and IL-1β was down-regulated (Cao et al., 2010). Of note, N-(N-(3,5-difluorophenacetyl)-lalany1)-S-phenyglycinet-butylester also inhibited the expression of NF-ϰB induced by hypoxia in microglia, indicating the interrelationship between Notch signaling and NF-ϰB pathways (Yao et al., 2013b). This corroborates with results of a separate study which reported that Notch and NF-ϰB pathways function synergistically in regulating several aspects of cellular functioning (Ang and Tergaonkar, 2007). The results with N-(N-(3,5-difluorophenacetyl)-lalany1)-S-phenyglycinet-butylester also indicate that the Notch pathway is located upstream of NF-ϰB and that it regulates microglia activation (Cao et al., 2008; Yao et al., 2013b).

### *Renin-angiotensin systems*

Renin-angiotensin system (RAS) is one of the neuroendocrine axes that regulate blood pressure, water, electrolytes and maintain the stability of the internal environment (Labandeira-Garcia et al., 2017). RAS is composed of renin, angiotensinogen, angiotensin and its cognate receptors whose existence has been reported in the developing brain (Millan et al., 1991; Tsutsumi et al., 1991). Angiotensinogen is the precursor of angiotensin II (AngII) and it serves as a substrate for renin and other enzymes capable of cleaving angiotensin (Benicky et al., 2009) from the N-terminal end. AngII is an effector molecule of RAS. Apart from regulating the blood pressure and fluid homeostasis, it also participates in a variety of processes such as cell growth, development of brain and cardiac development, atherothrombosis, inflammation and programmed cell death (Stoll and Unger, 2001). AngII performs multiple functions though linking to different receptor subtypes, namely, angiotensin II type 1 receptor (AT1) and/or angiotensin II type 2 receptor (AT2). It has been documented that AT2 may exert an opposing function to AT1 (Jones et al., 2008). It has been reported that AngII and its receptors AT1 and AT2 are localized in the amoeboid microglial cells in the developing brain. Of note, AngII and AT1/AT2 expression was increased following middle cerebral artery occlusion (MCAO) (Wu et al., 2013). In experimental cerebral ischemia model or experimental intracerebral hemorrhage model, it has been reported that inhibition of AT1 was able to down-regulate the release of TNF-α (Danielyan et al., 2007), reduce the infarct area (Nishimura et al., 2000), hemorrhage volume, brain edema, inflammatory response and apoptosis (Jung et al., 2007) alluding to the possibility that AT1 is involved in inflammatory response in brain injuries; in other words, AT1 plays an important role in brain injury (Benicky et al., 2009).

Relevant to the discussion of AT1 are sirtuins, which are NAD-dependent deacetylases whose enzymatic activity is regulated by the ratio of NAD to NADH. High NAD levels activate sirtuins and conversely high NADH levels inhibit their activity (Kincaid and Bossy, 2013; Lin and Guarente, 2003). Of the seven sirtuins, sirtuin 3 (SIRT3) is the only sirtuin analogue whose increased expression was shown to be associated with longevity of humans (Rose et al., 2003). SIRT3 is localized primarily in the mitochondria and has been shown to bind and deacetylate several metabolic and respiratory enzymes that regulate important mitochondrial functions (Onyango et al., 2002). There is evidence that SIRT3 can alleviate cell dysfunction caused by AngII (Liu et al., 2015) and regulate the production and clearance of reactive oxygen species (ROS) through deacetylation of numerous mitochondrial enzymes by multiple mechanisms (Ahn et al., 2008; Tao et al., 2010; Bell and Guarente, 2011; Bell et al., 2011). AngII via AT1 stimulation can activate NAD(P)H oxidase to produce ROS, resulting in oxidative stress damage (Griendling et al., 1994) that inhibits the expression of SIRT3 in mitochondria and ultimately leads to mitochondrial dysfunction and cellular damage (Gendron et al., 2003).

There is strong evidence that AngII can mediate several key events of the inflammatory processes. For example, AngII has been shown to activate NF-ϰB signaling pathway via AT1, which initiates the transcription of inflammatory cytokines, leukocyte adhesion and oxidative stress and impairs blood-brain barrier (BBB) integrity (Brouns and Deyn, 2009; Li et al., 2016). It stands to reason therefore that inhibition of the angiotensin converting enzyme (ACE) can reduce the formation of AngII and inhibit AngII/AT1 mediated production of proinflammatory factors (Wang et al., 2016). Very strikingly, as opposed to AT1, it has been reported that stimulation of AT2 activates multiple signaling pathways which are linked to beneficial effects on neuronal functions, including excitability, differentiation, and regeneration, inflammation, oxidative stress, and cerebral blood flow (Guimond and Gallo-Payet, 2012). Search and identification of natural compounds that can regulate AngII and its receptor protein expression in brain injuries therefore should be one of the future scopes of our study.

*Mammalian target of rapamycin (mTOR) signaling*

mTOR senses intracellular and extracellular signals, such as nutrient deficiency, involved in regulating cell growth and proliferation, as well as physiological processes such as transcription, mRNA turnover and translation, ribosomal biogenesis, vesicular trafficking, autophagy and cytoskeletal organization (Saxton and Sabatini, 2017). mTOR complex 1 (mTORC1) together with mammalian target of rapamycin (mTOR) complex 2 (mTORC2) is the central component of the mammalian target of rapamycin (mTOR) signaling pathway (Meng et al., 2018). The production of proinflammatory cytokines was inhibited by the overexpression of mTOR in freshly isolated human monocytes and primary myeloid dendritic cells. Thus, the mTOR pathway might play a negative regulatory role in the immune system (Thomson et al., 2009).

Phosphatase and tensin homolog (PTEN) is an upstream inhibitor of mTOR which is therefore well recognized as a prosurvival pathway, suggesting it as a prospective molecular target for anti-cancer research (Vazquez et al., 2000). Moreover, several studies have suggested that PTEN exerted anti-inflammatory activity (Lee et al., 2006; Tsoyi et al., 2009). Recent studies have indicated that mTOR selectively regulated microglia activation in response to noxious stimulus and plays a crucial role in microglia viability (Russo et al., 2009). For example, it mediated the induction of iNOS in the BV-2 microglia induced by hypoxia (Lu et al., 2006), and decreased the incidence of surviving neurons after brain injury (Erlich et al., 2007). Thus, it has been suggested that mTOR plays a key role in the control of microglial functions.

*NF-κB signaling and its "cross-talks" with other pathways*

It has been reported that hyperphosphorylation of MAPKs molecules has an effect on NF-κB activation and the subsequent production of inflammatory mediators (Guimarães et al., 2013). It is well recognized that NF-κB is maintained in a latent form in the cytoplasm where it is in complex with IκB. Many studies (Saccani et al., 2002; Cai et al., 2018, 2019) have reported that NF-κB is an important transcription factor involved in inflammatory processes as well as regulation of inflammatory factors such as cytokines, chemokines, inducible enzymes, growth factors and immune receptors (Nam, 2006).

IκB kinase complex (IKK) is required for activation of NF-κB; it contains the kinases IKKα, IKKβ or IKKγ and the regulatory NEMO (NF-κB essential modulator) subunit (Li et al., 2002). This is evident when cells are challenged with the proinflammatory cytokines and other stress-like stimuli. Although both IL-1β and TNF-α require NEMO for classical NF-κB activation, NEMO forms a functional IKK with IKKα in response to IL-1β, but TNF-α requires IKKβ to form a signaling unit (Solt et al., 2007). In addition to the above is Toll-like receptor 4 signaling that emanates from cytokine receptors and which acts as a physiological stimulus to induce activation of canonical NF-κB pathway. This process involves the phosphorylation of IκB and nuclear translocation of mostly p65-containing heterodimers (Oeckinghaus et al., 2011). Activated NF-κB initiates expression of a multitude of inflammatory response target genes such as TNF-α, IL-1β, IL-6, IL-8, iNOS and COX-2. (D'Acquisto et al., 2002). In this connection, increasing evidence supports the existence of important but poorly understood cross-talk between NF-κB and other signaling pathways in different cells, including macrophages and microglia (Cao et al., 2010, 2011; Oeckinghaus et al., 2011; Zhang et al., 2012).

A previous study has demonstrated that Notch can enhance NF-κB gene binding activity in microglia when challenged with LPS (Cao et al., 2010). It has been shown that Notch blockade can inhibit NF-κB/p65 expression and translocation into the nucleus in a hypoxic environment. It was speculated that some elements or factors which lead to the release and translocation of NF-κB/p65 might have been affected by Notch signaling blockade. However, because NF-κB activity is controlled at different levels by positive and negative regulatory elements, multiple targets may be involved in regulating the action of Notch signaling in NF-κB activity. To this end, one possible candidate to be considered is glycogen synthase kinase (GSK). GSK-3β, a multifunctional kinase, is regulated by serine (inhibitory) and tyrosine (stimulatory) phosphorylation (Grimes and Jope, 2001). GSK-3β expression is ubiquitous in eukaryotes and plays diverse roles in various essential physiological and pathological processes, including glycogen metabolism, cell cycle control, apoptosis, embryonic development, cell differentiation, cell adhesion and inflammation (Doble and Woodgett, 2003; Li et al., 2004; Wang et al., 2011a). Additionally, GSK-3β regulates toll-like receptor (Martin et al., 2005) and production of IL-6 after LPS stimulation (Ajmone-cat et al., 2016). GSK-3β mediates the release of IL-1β, TNF-α and IL-10 from cortical glia and microglia in response to LPS stimulation (Beurel and Jope, 2009). It has been reported that phosphorylation of GSK-3β was associated with Notch-1 activity (Cao et al., 2017). Meanwhile, Notch-1 is reciprocally regulated by GSK-3β. This is evident by the decreased expression of NICD and hairy and enhancer of split-1 (Hes-1) by LiCl, a GSK-3β inhibitor that binds to the magnesium-sensitive site of the enzyme (Shahal et al., 1996; Stambolic et al., 1997). Both GSK-3β and Notch-1 can regulate activation of microglia (Koistinaho et al., 2011; Gentle et al., 2012). It is interesting to note that GSK-3β plays a role in modulating the NF-κB/p65 and IκB-α. Blocking Notch-1 by N-(N-(3,5-difluorophenacetyl)-lalany1)-S-phenyglycinet-butylester also upregulated IκB-α (Cao et al., 2017). Taken together, it is suggested that Notch-1, NF-κB/p65 and GSK-3β operate in synergy to mediate microglia activation. All in all, it can be confidently concluded

from the available evidence that the three signaling pathways are functionally interrelated in regulating microglia activation.

In addition to the above-mentioned signaling pathways, another signaling pathway worthy of consideration is the G protein-coupled estrogen receptor 1 (GPER), a 7-transmembrane receptor, which is highly expressed in many brain regions such as dentate gyrus, CA1 and CA3 regions. GPER was first reported to have an estrogen binding affinity which exerted neuroprotective and anti-inflammatory actions (Revankar et al., 2005). In the rapid estrogen receptor signaling pathway, GPER activation is associated with phosphorylation of MAPKs extracellular signal-regulated kinase (ERK)1/2 as well as nuclear translocation of NF-κB, which leads to the activation of adenylate cyclase, phospholipase C (PLC), and growth factor receptor (Luo et al., 2012; Bean et al., 2014; Zhu et al., 2017) and plays an important anti-inflammatory and anti-apoptotic role (Tamaki et al., 2014; Zhao et al., 2016). Very interestingly, G1, a GPER selective agonist, can inhibit the activation of NF-ϰB in LPS-induced microglia activation (Gao et al., 2019). Concomitantly, G1 attenuates neuroinflammation and dopaminergic neurodegeneration in PD (Guan et al., 2017).

One member of the family of phosphoinositide specific PLCs (PI-PLC)-PLC-γ can convert phosphatidylinositol 4,5-bisphosphate to 1,2-diacylglycerol and inositol 1,4,5-trisphosphate. There are two forms of PLC, namely, PLC-γ1 and PLC-γ2. PLC-γ1 is widely distributed in different tissues and cells and plays an essential role in development in mammals. PLC-γ1 deficient mice may develop normally but die soon after embryonic day 8.5 (Ji et al., 1997). PLC-γ1 plays a key role in growth factor-dependent signal transduction (Patterson et al., 2005). There is increasing evidence suggesting that PLC-γ1 can protect the apoptosis of neurons induced by oxidative stress (Lee et al., 1999; Hayashi et al., 2009; Yuan et al., 2009). Recently, some studies suggested that PLC-γ1 deficiency caused inflammatory and autoimmune symptoms and abrogated the anti-inflammatory effects of lipoxins on endothelial cell inflammation to block the development and function of Treg cells (Baker et al., 2009; Fu et al., 2010). Separately, and in our own studies, we found that the PLC-γ1 pathway was linked to regulating expression of NF-ϰB/RelA, phosphorylation of IϰB and cAMP responsive element binding (CREB) in LPS-stimulated microglia, which protected against LPS-induced neuroinflammation. Of note, some natural compounds were found to possess anti-inflammatory properties that can alleviate the inflammatory response in the brain by targeting PLC-γ1 in microglia activation (Zong et al., 2012).

Inhibition of NF-ϰB mediated microglial activation attenuates neuronal damage and improves memory impairment *in vitro* and *in vivo* (Ghosh et al., 2007; Lee et al., 2015; Liang et al., 2018). NF-ϰB is potentially an important molecular target for the development of agents against inflammatory diseases and many recent studies have focused on the regulation of NF-ϰB. It is relevant to note that the NF-ϰB activity is closely related to that of the MAPKs signaling pathway (Zhou et al., 2008) as discussed below.

### *MAPKs signaling*

MAPKs are a family of serine/threonine protein kinases that are involved in various cellular responses such as cell proliferation, differentiation, and apoptosis. MAP kinases are key mediators of eukaryotic transcriptional responses to extracellular signals and control gene expression via the phosphorylation and regulation of transcription factors, co-regulatory proteins and chromatin proteins (Whitmarsh, 2007). The MAPKs family comprises three members which are the ERK, c-Jun N-terminal kinase (JNK), and p38 MAPK; each member exerts different biological functions (Wang et al., 2007). ERK1/2 is activated by MAPK kinase (MKK) 1 and MKK2, c-Jun N-terminal kinases (JNK) by MKK4 and MKK7, while p38 MAPK kinase is activated by MKK3, MKK4, and MKK6. The activation of MAP kinase leads to the activation of transcription factors, which leads to the expression of target genes and triggers a biological response. Multiple interactions among cascades of different MAP kinases can integrate reactions and activate different sets of genes (Pearson et al., 2001; Karin and Gallagher, 2009). The activated ERK1/2 MAPK pathway takes part in cellular proliferation and survival; however, c-Jun N-terminal kinases (JNK) and p38 MAPK partake in cellular apoptosis (Kim and Choi, 2010). Relevant to the present discussion are p38 MAPK and ERK which appear to be primarily involved in the production of proinflammatory mediators in activated microglia. Studies have shown that MAPKs are involved in regulating the expression of iNOS, TNF-α and IL-1β in activated microglia.

As a downstream target of MAPK, CREB plays an important role in the development of neuropathic pain by regulating transcription and secretion of diverse neurotransmitters. Binding of cAMP to the regulatory subunit of protein kinase A phosphorylates CREB, which eventually regulates multiple cellular events. Previous studies have shown that activated p38 or ERK1/2 induces CREB phosphorylation in microglial or neuronal cells (Canon et al., 2004; Li et al., 2017). Consistent with this, isotalatizidine, a C19-diterpenoid alkaloid can promote the expression of microglial dynorphin A by stimulating the ERK/CREB signaling pathway.

MAPK phosphatases primarily terminated signal transduction and deregulation via a group of 11 dual-specificity phosphatases dephosphorylating the MAPKs on their threonine and tyrosine residues (Whitmarsh, 2007). Blocking of MAPK inhibited the mRNA expression of IL-1β and TNF-α in ischemic brain in rat and in LPS stimulated microglia (Zawadzka and Kaminska, 2005). The inhibitor of MAPK reduced the

levels of IL-1β and TNF-α mRNAs in cultured cortical astrocytes under basal conditions (Zawadzka and Kaminska, 2005).

It has been reported that the inhibition of MAPK pathways has a therapeutic effect on the process of inflammatory responses. Therefore, it would be desirable to search for agents that can inhibit MAPK pathways effectively and safely, notably in the activated microglia in brain injuries or diseases.

### *microRNA*

In addition to altered signaling pathways, activated microglia in different pathological conditions exhibit dysregulated epigenetic mechanisms, such as chromatin modifications, changes in gene-specific histone acetylation and methylation, as well as differential microRNA (miRNA) expression (Jadhav et al., 2014; Patnala et al., 2017). As an endogenous single stranded non-coding RNA, miRNAs mediate specific gene post-transcriptional regulation. If the process of regulation is abnormal or dysfunctional, it may involve multiple pathological processes of many diseases (Wang et al., 2013). Thus, miRNA dysregulation in activated microglia is reported in the occurrence and development of neurodegenerative diseases and progression of brain injuries (Karthikeyan et al., 2016). A global miRNA microarray analysis identified several miRNAs which were differentially expressed in LPS and IL-4 stimulated microglia. For example, miR-146a, a well-studied regulator of innate immune responses, has been found to possess anti-inflammatory effects; it targets TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor associated kinase-1 and IL-1 receptor associated kinase-2 genes. MiR-146a overexpression leads to suppression of cellular inflammatory response and a decrease in cytokine secretion. It was enriched in activated macrophages and microglia (Aronica et al., 2010) and has been shown to target and suppress mediators of the NF-ϰB signaling pathway, which might compensate for excessive inflammation to restore homeostasis (Gaudet et al., 2018).

TRAF6, as a downstream protein of Toll-like receptor 4 signaling, plays a critical role in the activation of subsequent inflammatory responses, such as the NF-ϰB signaling pathway (Cui et al., 2018; He et al., 2018). A previous study has suggested that inhibiting TRAF6 ubiquitination ameliorates brain inflammatory injury (Dou et al., 2018).

*In vivo* and *in vitro* experiments confirmed that multiple pro-inflammatory mediators can induce immediate expression of miR-146a-5p, which is an early-response gene (Gaudet et al., 2018). In LPS induced BV2 and N9 microglial cells and hippocampal tissues of mice with surgical trauma, expression of miR-146a-5p was increased (Gaudet et al., 2018). The expression of miR-146a-5p rose sharply when BALB/c mice brain and mouse microglial C8-B4 cells were challenged with Japanese encephalitis virus (Sharma et al., 2015). In peritoneal macrophages (Pan et al., 2018), bronchial epithelial cells (Lambert et al., 2018), bone marrow-derived mesenchymal stem/stromal cells (Kubota et al., 2018), astrocytes (Lu et al., 2015), BV2 microglial cells (Karthikeyan et al., 2016) and human brain microglial cells (Sharma et al., 2015) miR-146a exerts its anti-inflammatory effects. Exogenous supplementation of synthetic miR-146a modulated inflammatory cytokines such as TNF-α, COX-2, iNOS, IL-6, IL-8, RANTS and the ion channel, the transient receptor potential channel vanilloid 1 in human glial cells (Li et al., 2011). Intrathecal miR-146a-5p mimic attenuated spinal nerve ligation-induced mechanical allodynia and decreased spinal TRAF6 expression (Lu et al., 2015). Overexpression of miR-146a-5p inhibited inflammatory response in BV2 cells (Ge et al., 2019). In addition, miR-146a was reported to target Notch1 in glioma cells and inhibit gliomagenesis by suppressing migration and proliferation of cancer cells (Mei et al., 2011). Bioinformatic analysis by us had predicted miR-146a targeting SMAD4 thus supporting its role in regulating microglia activation and gliomagenesis (Karthikeyan et al., 2018).

## Effects of natural compounds on activated microglia and mediated neuroinflammation

### *Resveratrol*

Resveratrol (3,4,5-trihydroxy-trans-stilbene), a natural nonflavonoid polyphenolic is abundant in grapes, red wine, mulberries, knotweed, etc. Baur and Sinclair first described resveratrol as a phenolic component of the medicinal herb hellebore (Baur and Sinclair, 2006). Its chemical structure is two phenolic rings connected by a double bond (Shakibaei et al., 2009). There are two isoforms of resveratrol: trans-resveratrol and cis-resveratrol in which the trans-isomer is more stable and recognized to have greater biological activity when not in contact with high pH and UV light (Athar et al., 2007). Resveratrol is absorbed mainly in the duodenum as shown from studies in rat intestines. Studies with radio-labeled resveratrol in mice have demonstrated that resveratrol can be detected in the lung, spleen, heart and brain by 3h post-administration (Vitrac et al., 2003). It is widely reported that resveratrol exerts multiple biological effects such as anticancer, anti-inflammatory and antioxidant activities (Shakibaei et al., 2008; Imler and Petro, 2009). Additionally, resveratrol can exert a neuroprotective effect against ischemia, seizure and neurodegenerative disorders (Markus and Morris, 2008). This is because reveratrol has the ability to cross the BBB after intraperitoneal administration (Baur and Sinclair, 2006). There is emerging evidence that many alternative and nutrition therapies can modulate the immune system and disrupt the proinflammatory cascade through a variety of mechanisms, including antioxidant effects, alterations in cell signaling, cytokines and proinflammatory mediators. In this connection,

resveratrol plays a potentially important role in the prevention and treatment of various neuroinflammation-related diseases such as AD, PD, Huntington's disease, autism spectrum and neuropathic pain (Anekonda, 2006). This is because resveratrol has been shown to possess anti-oxidant and anti-inflammatory effects and that many signaling pathways are among its molecular targets. In light of this, voluminous *in vivo* and *in vitro* studies on resveratrol have been carried out in the past decades to provide a theoretical basis for its clinical application.

Indeed, a preclinical study reported the protective effects of resveratrol with 12 weeks of oral administration on aging-induced cognitive impairment (Gocmez et al., 2016). In addition, a clinical trial for AD patients showed that long term oral treatment with resveratrol for 53 weeks effectively decreased the level of pro-neuroinflammatory factors in cerebrospinal fluid and improved cognitive function (Turner et al., 2015; Moussa et al., 2017). Furthermore, it has been shown that resveratrol can alleviate amyloid peptide-induced neurotoxicity (Huang et al., 2011), promote clearance of Aβ and reduce senile plaques (Karuppagounder et al., 2009) in cell or AD mouse models (Robb et al., 2008). In a rat model of AD, it has been demonstrated that treatment with resveratrol was able to confer a significant improvement in spatial memory. The neurological protection effects of resveratrol seemed to be associated with the reduction of iNOS and lipid peroxidation in the cell and by promoting the production of heme oxygenase-1 (Huang et al., 2011). In postoperative cognitive dysfunction, a consequence of acute neuroinflammatory response triggered by surgical procedure (Kawano et al., 2018), nanoemulsion loaded with resveratrol was found to improve the cognitive decline that followed (Locatelli, 2018). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that induces Parkinsonian features in humans, rodents and non-human primates and has been demonstrated to cause rapid and selective dopamine neurotoxicity (Lofrumento et al., 2014). MPTP also leads to a sustained inflammatory response in both humans and primates over a prolonged duration. MPTP also modifies the expression of numerous proinflammatory genes including IL-1β, IL-6, IL-10 and TNF-α (Lofrumento et al., 2014). Blocking microglia inflammatory response caused by MPTP may offer some neuroprotection. It has been demonstrated that resveratrol has a neuroprotective role in MPTP-induced parkinsonism by scavenging radicals, thus significantly alleviating MPTP-induced motor coordination impairment, hydroxyl radical overload and neuronal loss (Lofrumento et al., 2014) in mice. Separately, in a MPTP-based PD mouse model, it was observed that resveratrol treatment significantly reduced glial activation, the level of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and their respective receptors (IL-1RI, TNF-RI, IL-6R) in the SNpc of mice. Consistent with these observations, resveratrol treatment enhanced the expression of suppressor of cytokine signaling-1, thus supporting the notion that resveratrol protects SNpc dopamine neurons against MPTP-induced cell death by modulating inflammatory reactions (Lofrumento et al., 2014).

In the CNS, diseases including traumatic brain injury and cerebral ischemia, resveratrol has been shown to have promising neuroprotective effects due to its multiple functions (Lin et al., 2014). Traumatic brain injury leads to sustained microglia activation over a protracted period after the initial impact, thus generating an array of proinflammatory cytokines which can cause the axonal injury as observed during the chronic phase of secondary brain injury (Gentleman et al., 2004). High dose resveratrol treatment after mild traumatic brain injury resulted in attenuation of neuroinflammation in which microglia activation was inhibited and production of proinflammatory cytokines, such as IL-6 and IL-12 was decreased (Gatson et al., 2013). Furthermore, recent studies have also demonstrated that resveratrol protected against early brain injury after subarachnoid hemorrhage due to its powerful anti-inflammatory properties (Zhang et al., 2016).

Recent studies have shown that resveratrol exerted a dose-dependent cerebroprotective activity against cerebral ischemia and reperfusion (I/R)-induced injury in rats. Resveratrol is endowed with antioxidant and anti-inflammatory properties in reduction of oxidative stress and inflammatory mediators, such as TNF-α, IL-6 and intercellular cell adhesion molecule-1, which are implicated in cerebral infarction mediated by I/R (Orsu et al., 2013). It has also been reported that resveratrol treatment is neuro-protective of the hippocampal neurons of young adult rats with induced acute bacterial meningitis (Sheu et al., 2013).

It is now widely accepted that resveratrol plays a protective role in multiple pathological conditions and that this is due to its powerful anti-inflammatory and antioxidant properties. Because activated microglia are the key players in neuroinflammation implicated in different brain injuries or diseases, it is therefore reasonable to suggest that resveratrol would have a direct effect on them. It is conceivable that this would involve the regulation of multi-signal networks that drive the microglial activation. Indeed, resveratrol has been reported to inhibit LPS-induced NO and prostaglandin E2 production by rat astroglioma cells as well as inhibit TNF-α and iNOS expression, and NO production in a mouse microglial cell line (Lawrence et al., 2001; Bi et al., 2005). In addition to decreasing the level of LPS-stimulated TNF-α and NO, resveratrol also exerted in a dose-dependent manner the expression of IL-1β, IL-6 and monocyte chemoattractant protein 1 (MCP-1) in LPS-stimulated primary microglia and N9 microglial cells (Lu et al., 2010).

It was reported that resveratrol preferentially inhibited NF-κB activation by interfering with IKK and IκB phosphorylation and reduced the transcriptional stimulation of several NF-κB target genes, including

TNF-α and IL-6 when treated with the Toll-like receptor 4 ligand-LPS in BV-2 microglia. Concomitant to this, downstream phosphorylation of signal transducer and activator of transcription 1 and signal transducer and activator of transcription 3 following LPS stimulation was also inhibited by resveratrol (Capiralla et al., 2012). As a result, the expression of NO, prostaglandin E2, TNF-α and iNOS was decreased; however, the upstream regulation mechanism of resveratrol needs to be clarified.

In a previous study by us (Zhong et al., 2012), it was found that resveratrol targeted the mTOR signaling pathway to inhibit inflammatory response in cultured microglial cells challenged with LPS. Separately, in freshly isolated human monocytes and primary myeloid dendritic cells, activation of mTOR down-regulated the production and release of proinflammatory cytokines by blocking NF-κB and signal transducer and activator of transcription 3 activity (Weichhart et al., 2008). It is therefore suggested that the mTOR pathway might be involved in the immune regulation of the nervous system (Thomson et al., 2009). We have shown that resveratrol can activate the mTOR signaling pathway and affect LPS-induced mTOR phosphorylation. Remarkably, when the cells were treated with resveratrol (50 μM) for 1h before LPS (1 μg/ml) stimulation, the phosphorylation of mTOR, PTEN and AKT, the effective modulator of mTOR, was significantly higher compared with the cells treated with LPS only.

We have demonstrated that resveratrol promoted inactivation of PTEN and increased phosphorylation of Akt in LPS-stimulated BV-2 cells. Thus, this suggested that the modulatory effect of resveratrol on inhibition of expression of proinflammatory mediators is closely related the regulation role of PTEN/Akt/mTOR signaling in LPS-activated BV-2 cells (Zhong et al., 2012). We concluded that mTOR signaling is involved in resveratrol-inhibited release of NO, prostaglandin E2, iNOS, COX-2, TNF-α and IL-1β induced by LPS in BV-2 microglia.

A variety of transcription factors, including NF-κB and CREB, are known to be involved in the transcriptional regulation of the abovementioned inflammatory mediators (Vaillancourt et al., 2007). The underlying molecular mechanism of neuroprotective effect of resveratrol is its potent anti-inflammatory activity. We have shown that NF-κB/RelA level was markedly increased by LPS stimulation in BV-2 cells and that it was effectively reversed by resveratrol treatment in a mTOR-dependent manner (Zhong et al., 2012). The mTOR pathway is linked to resveratrol-suppressed LPS-induced expression of NF-κB/RelA protein, phosphorylation of IκB-α and CREB, and protects against LPS-induced activation in BV-2 cells. CREB is the physiological substrate for MAPKs and stress activated protein kinases-1, which are activated by ERK and p38 MAPK-mediated signaling in response to LPS. The inhibitory effect of resveratrol on MAPKs was dependent on mTOR signaling during BV-2 cells activation induced by LPS.

MicroRNA-146a (miR-146a), a well-studied regulator of innate immune responses, has been reported to possess anti-inflammatory functions in BV-2 microglia (Juknat et al., 2019) and primary astrocytes (Lu et al., 2015). MiR-146a was increased in some preclinical models such as stroke (Liu et al., 2017), ischemia-reperfusion injury (Wang et al., 2013), epilepsy (Aronica et al., 2010) and neuropathic pain (Lu et al., 2015). It is induced by NF-κB activation and forms a regulatory negative feedback loop in monocytes (Gaudet et al., 2018). Importantly, resveratrol enhanced the expression of miR-146a-5p, whereas the anti-inflammatory functions of resveratrol were attenuated by miR-146a-5p silence via its specific inhibitor in microglia.

Interestingly, the suppression of the TRAF6/NF-κB signaling pathway induced by resveratrol in LPS stimulated BV2 cells was partially reversed by miR-146a-5p silence. Collectively, these data suggested that interaction between miR-146a-5p and the TRAF6/NF-κB signaling pathway was implicated in the protective functions of resveratrol on LPS-stimulated BV2 cells. All in all, it can be concluded that resveratrol ameliorated inflammatory injury by inactivating the TRAF6/NF-κB pathway, which might be modulated by miR-146a-5p (Ge et al., 2019). The findings have demonstrated unequivocally the apparent anti-inflammatory mechanism of resveratrol in activated microglia.

### *Ginsenosides*

Among the various Chinese herbal compounds, Ginsenosides ("Ren Shen") found in plants of the genus Panux and the pharmacologically active components in ginseng are probably the most widely investigated natural product. Ginsenosides are a triterpenoid saponin compound with a four ring hydrophobic steroid structure and sugar moieties (monomeric, dimeric or trimeric) attached mainly at carbons 3, 6 and 20 (Zhu et al., 2004; Nah et al., 2007). Ginsenosides are divided into two different structural classes: the 20 (S)-protopanaxadiol including ginsenosides Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Rc, Rd, Rg3 and Rh2 and the 20 (S)-protopanaxatriol including ginsenosides Re, Rf, Rg1, Rg2, and Rh1 due to the positioning of glycoside attachments on the molecules (Zhu et al., 2004; Nah et al., 2007; Baek et al., 2012). The most commonly explored ginsenosides include Rb1, Rg1, Rg3, Re and Rd. In general, intact ginsenosides are metabolized in the stomach by acid hydrolysis and absorbed in the intestines by bacterial hydrolysis (Hasegawa et al., 1997; Hasegawa and Uchiyama, 1998; Tawab et al., 2003; Hasegawa, 2004; Bae et al., 2006; Hou et al., 2012; Jung et al., 2012; Zhao et al., 2012). All evidence seems to converge that it is important for ginsenosides to be metabolized and transformed in intestines. It has been reported that glycosylated-ginsenosides take off the sugar moieties,

becoming deglycosylated ginsenosides, which are more permeable, bioactive (Hasegawa and Uchiyama, 1998) and absorbable into the bloodstream and play important roles in anti-tumor (Xu et al., 2007; Lee et al., 2009), antioxidant, anti-inflammatory (Keum et al., 2003; Bae et al., 2006), anti-fatigue and angio-suppressive effects (Yue et al., 2006) in the body. Thus, ginseng is a widely recognized natural compound which is good for the health, fostering immune function, increasing resistance to stress, and improving cardiac function (Kiefer and Pantuso, 2003).

In neurological disorders, ginsenosides have attracted the attention of many studies in recent years. They have been investigated as neuroprotective agents for PD and among them Rb1, Rg1, Rd and Re may be mentioned specifically. They have been shown to exert neuroprotective effects by inhibiting oxidative stress and neuroinflammation, decreasing toxin-induced apoptosis and nigral iron level, regulating activity of N-methyl-D-aspartate receptor channel and targeting a-synuclein abnormalities in the substantia (Heng et al., 2016). In MPTP-induced PD mouse model, Rg1 has immunomodulation effects (Zhou et al., 2015). In ischemia-reperfusion injury, ginsenoside Rg1 is evidently neuroprotective as it blocks calcium influx into neurons, decreases nNOS activity and modulates aquaporin 4 expression and BBB disruption (He et al., 2014; Zhou et al., 2014).

Additionally, ginsenosides are reported to have antioxidant effects and modulate Aβ 25-35 induced mitochondrial dysfunction (Yan et al., 2013). Treatment with ginsenoside Rg1 significantly decreases the level of Aβ in cerebral tissue, reverses neuropathological changes and protects spatial learning abilities and memory (Fang et al., 2012) in transgenic AD mice overexpressing APP/Aβ and the memory impairments in rats induced by okadaic acid (Song et al., 2013). Meanwhile, ginsenoside-Rg1 has neuroprotective effects on depression-associated disorder involved in modulation of inflammatory response, synaptic deficits and neuronal apoptosis. It is also noteworthy that chronic administration with ginsenoside-Rg1 prior to stress exposure significantly alleviated the overexpression of proinflammatory cytokines and activation of microglia and astrocytes. Moreover, ginsenoside-Rg1 inhibited neuronal apoptosis and attenuated dendritic spine and synaptic deficits induced by chronic unpredictable mild stress exposure in the ventral medial prefrontal cortex (Fan et al., 2018). Taken together, it is safe to conclude that Rg1 is neuroprotective because it has been widely shown to be involved in antioxidant and anti-inflammatory process.

The next thing needing consideration is the underlying mechanism whereby Rg1 can exert its anti-inflammatory role in the CNS, notably its effects on regulation of the activated microglia who are the key player cells in neuroinflammation (Aguzzi et al., 2013). Studies have found that almost all forms of ginsenosides can suppress TNF-α production but NO production in microglia was inhibited by ginsenoside-Rg1 and -Re in LPS activated N9 microglial cells (Wu et al., 2007).

In a previous study, we reported that Rg1 suppressed cyclophosphamide-induced elevation of the proinflammatory cytokines, TNF-α and IL-6; meanwhile, it increased the levels of anti-inflammatory cytokines IL-4 and IL-10 in sera and brain tissues (Shi et al., 2019). Rg1 also modulated cytokine mediators and inhibited cyclophosphamide-induced microglial polarization from M2 to M1 phenotypes. In a model of neuroinflammation induced by cyclophosphamide *in vitro*, Rg1 co-treatment strikingly reduced cyclophosphamide's neurotoxic effects and inflammatory response, not only in PC12 neuroblastic cells but also in hyperactivation of BV-2 microglial cells. These results indicate that Rg1 can exert its anti-inflammatory effects by modulating cytokines and the related upstream mediators in microglia to play a role in protecting neuronal activity and promoting neuroplasticity, in particular brain regions associated with cognition processing (Shi et al., 2019).

We have also reported that ginsenoside Rg1 suppresses expression of iNOS, COX-2, IL-1β and TNF-α not only at mRNA level but also at protein level in LPS-stimulated BV2 microglia (Zong et al., 2012). Rg1 significantly suppressed LPS-induced iNOS promoter activity by inhibiting DNA binding of transcription factor NF-κB, and its upstream signaling molecules, such as phosphorylation of IκB and CREB. A pivotal role for three MAPK members in signaling transduction of microglial cells was identified in LPS induced NF-κB activation and subsequent iNOS and COX-2 production (Ock et al., 2009). As expected, LPS increased activation of MAPKs, including ERK1/2, c-Jun N-terminal kinases (JNK) and p38 MAPK. Remarkably, Rg1 decreased the LPS-induced activation of MAPKs along with reduced expression of iNOS, COX-2 and proinflammatory cytokines. Subsequent exploration confirmed that the inhibitory process of Rg1 on microglia activation is mediated by PLC-γ1. PLC-γ1 blockage abolished the role of Rg1 on regulating the iNOS, COX-2, IL-1β and TNF-a protein and mRNA expression. The findings strengthened the notion that PLC-γ1 participates in anti-inflammatory effects and modulates microglia activation in the CNS (Zong et al., 2012).

Despite the above findings, it is clear that regulation of Rg1 on activated microglia remains to be fully explored in view of the complex process of microglia activation. The involvement of GPER in the anti-inflammatory effects of ginsenoside Rg1 against LPS-induced microglia activation in BV2 microglia and ventral mesencephalic primary microglial culture was thus considered. The GPER mediates the non-genomic effect of estrogen and plays an important role in the anti-inflammatory and anti-apoptotic effects of estrogen. Both Rg1 and GPER-specific agonist G1 treatment significantly inhibited the LPS-induced activation of the NF-κB and MAPK signaling pathways in microglial cells. Interestingly, GPER antagonist G15 blocked the

inhibitory effects of Rg1 and the GPER-specific agonist G1 on LPS-induced microglia activation. Activation of GPER can regulate the activation of NF-κB and MAPK signaling pathways; hence, it may be the upstream of NF-κB. Inhibition of GPER by pharmacological blockade and lentivirus mediated siRNA knockdown of GPER abrogated the anti-inflammatory effects of Rg1 on regulation of NF-κB and MAPK signaling pathways and proinflammatory cytokines, including iNOS, COX-2, IL-1β and TNF-α. Taken together, the results indicate that Rg1 exerts its anti-inflammatory effects via GPER in LPS-induced microglia activation. These findings provide a new biological target of Rg1 for the treatment of neuroinflammatory disorders (Gao et al., 2019).

### *Gastrodin*

In addition to ginsenosides, the search for other herbal compounds has been the focus of many studies in recent years with the aim of identifying potential candidates that may help ameliorate microglia mediated neuroinflammation under different neuropathological conditions. Among the variety of potential herbal agents is Gastrodin, the major bioactive constituent of the traditional Chinese herb "Tian ma" (Gastrodia elata Blume), which has been identified as a potential candidate. Gastrodin has attracted much attention because of its widespread application for centuries in the local communities for treatment of various neurological diseases, such as headaches, dizziness, vertigo and convulsive illnesses such as epilepsy and tetanus (Yang et al., 2007). Gastrodin (4-(hydroxymethyl) phenyl-β-D-glucopyranoside), with a simple chemical structure, is a water-soluble compound with a low molecular weight (286.27) (Cai et al., 2008). After oral administration, the peak concentration time (Tmax) of Gastrodin in plasma occurs within 45 min and rapidly decays after 1h, indicating that it is not stored in the body; it can be readily absorbed in the intestinal tract and exerts a rapid effect (Song et al., 2017). It has been reported that if Gastrodin was co-administered with borneol, it may be absorbed more readily from the gastrointestinal tract; meanwhile, the Tmax of Gastrodin in the plasma became shorter (5-15 vs. 30 min). Oral administration of Gastrodin sustained-release tablets, 150 mg twice daily for 28 days, significantly increased the rate of regulatory T cells (Treg)/Th17 cells in peripheral blood in patients with a high risk of transient ischemic attack, suggesting its potential inhibitory effect on the autoimmune inflammatory response in cerebral ischemia (Yang et al., 2013).

Gastrodin is rapidly metabolized into gastrodigenin (p-hydroxybenzyl alcohol) which binds to benzodiazepine receptor and plays a role in regulating the CNS (Lin et al., 2008). The bioavailability of gastrodigenin in the brain may increase substantially by 33.6-108.8% and exhibit brain-targeting effects (Cai et al., 2008). There is mounting experimental evidence indicating the multiple activities of Gastrodin, including anti-oxidant (Ha et al., 2001), anti-inflammatory (Kim et al., 2012), anti-epileptic (Hsieh et al., 2007), anti-obesity (Park et al., 2011), anti-anxiety (Jung et al., 2006) and improvement of learning and memory (Niu et al., 2004). Previous studies have demonstrated that, after systemic administration, Gastrodin can pass through the BBB and gain access to different areas of the brain tissues to play a protective role in different pathological conditions (Lin et al., 2007a, 2008). It has been demonstrated that Gastrodin acts as an analgesia in diabetes-related neuropathic pain (Sun et al., 2012a) and inflammatory pain (Xiao et al., 2016). The role of Gastrodin in decreasing the level of lipid peroxidation (Yong et al., 2009) and increasing the expression of antioxidant proteins genes depends on its activities of scavenging free radicals and antioxidant activity (Yu et al., 2005). When administered via the abdominal aorta, Gastrodin can alleviate spinal cord ischemia reperfusion injury by promoting mitochondrial anti-oxidant capacity, increasing the release of brain-derived neurotrophic factor and inhibition of the proinflammatory mediators (Fang et al., 2016). Gastrodin can dilate the cerebral blood vessels, increase cerebral blood flow, alleviate oxidative stress, improve learning ability and consolidate the memory condition in chronic cerebral hypoperfusion (Li and Zhang, 2015). Gastrodin can significantly improve neurological function, effectively reduce the infarct size and brain water contents and has significant protective effects against cerebral I/R injury (Liu et al., 2016). In 6-hydroxydopamine-hemiparkinsonian animals, Gastrodin pre-treatment improved motor function. Additionally, Gastrodin reversed the production of malondialdehyde and NO to abolish oxidative/nitrosative stress by increasing the level of total antioxidant capacity in the SNpc (Haddadi et al., 2018). Therefore, Gastrodin possesses a therapeutic effect in 6-hydroxydopamine induced PD rat model by improving behavioral abnormalities, decreasing the loss of nigral tyrosine hydroxylase-positive cells and inhibiting nigral TNF-α expression under oxidative stress conditions. The loss of substantia nigral dopaminergic neurons and microglial activation is simultaneous in PD rats induced by rotenone. The inflammatory mediators e.g. TNF-α and IL-1β, are involved in the substantia nigral damage. Gastrodin could suppress the expression of IL-1β and neuroinflammatory response for protecting dopaminergic neurons in the substantia nigra.

Despite the ample studies on Gastrodin using different experimental cell and animal models and approaches, it is surprising that there is a paucity of information on the effects of Gastrodin on activated microglia (Dai et al., 2011); indeed, this issue has remained elusive. It is clearly desirable to gain a better understanding of the underlying mechanism of Gastrodin on activated microglia, especially since the latter are now implicated virtually in all major neurological diseases. We first reported that Gastrodin significantly inhibited the protein and mRNA expression of iNOS,

COX-2, TNF-α, and IL-1β in a dose-dependent manner in LPS-stimulated BV-2 microglia (Dai et al., 2011), suggesting possible beneficial effects of Gastrodin by attenuating the activation of microglial cells.

The underlying molecular mechanisms of the anti-inflammatory effect of Gastrodin were further studied by us (Dai et al., 2011). We reported that Gastrodin inhibited the expression of NF-κB/RelA protein induced by LPS. Gastrodin suppressed the LPS induced phosphorylation of IκB-α and CREB. These results strongly indicate that the inhibition of Gastrodin on the expression of iNOS, COX-2 and proinflammatory cytokines is partially through regulating the expression of NF-κB/RelA and the phosphorylation of IκB-α and CREB in LPS-stimulated BV-2 cells.

Additionally, the effect of Gastrodin on activation (phosphorylation) of three MAPKs induced by LPS in BV-2 microglia was further confirmed. The results first indicated that Gastrodin exerts its anti-inflammatory actions by regulating the NF-κB signaling pathway and phosphorylation of MAPKs. The inhibitory effects of Gastrodin on the inflammatory response in different signaling routes are therefore unequivocal, but the actual mechanistic link between them remains to be fully elucidated.

Another signaling to be considered is RAS which has been reported to regulate microglia activation (Labandeira-Garcia et al., 2017). RAS, including renin, angiotensinogen, angiotensin and its receptors has been reported to be present in the fetal and neonatal brain for more than 30 years (Millan et al., 1991; Tsutsumi et al., 1991). It is apparent that AngII has diverse functions in regulating a variety of processes such as cell growth, brain and cardiac function, atherothrombosis, inflammation and programmed cell death (Stoll and Unger, 2001) through two different receptor subtypes, namely, AT1 and/or AT2. Experimental evidence indicates that RAS can exert an important role in hypoxic/ischemic or hemorrhagic brain injury (Liu et al., 2018). Thus, blocking AT1 inhibited the release of TNF-α and reduced the infarct volume in an experimental cerebral ischemia model (Nishimura et al., 2000; Danielyan et al., 2007). Previous studies reported the involvement of AngII and receptors in microglia activation that may contribute to the neuropathological development (Zawada et al., 2015). Expression of ACE, AT1, NADPH oxidase-2, iNOS and TNF-α was markedly attenuated by Gastrodin in hypoxic-ischemia brain damage in postnatal rats and in cultured BV-2 microglia challenged with LPS (Liu et al., 2018). Of note, Gastrodin enhanced the protein expression of AT2 and SIRT3. Evidence that Gastrodin can exert its anti-inflammatory effects against the hypoxic-ischemia brain damage by regulating the expression of the RAS system (except for AT2 and SIRT3) in microglia has added a new perspective to its therapeutic potential.

Moreover, recent studies have suggested the involvement of Wnt/β-catenin signaling in generating inflammatory responses in the CNS through glial cell modifications (Marchetti and Pluchino, 2013). As the core regulator of Wnt signaling, β-catenin accumulates in microglia in AD and in an age-dependent way. Studies have shown that β-catenin was stabilized and enhanced in the cytosol in activated microglia in the inflamed brain; its nuclear import in proinflammatory microglia transformation is implicated under conditions of chronic neuroinflammation (Halleskog et al., 2011). GSK-3β is also involved in a variety of biological functions, including regulation of cell division, differentiation, cell migration and apoptosis. Recently, GSK-3β was identified as a crucial regulator of the inflammatory response (Beurel et al., 2010). We have shown that in LPS-induced microglia activation, the expression level of β-catenin and p-GSK-3β was drastically increased, which can be effectively inhibited by Gastrodin (Yao et al., 2019). It was suggested that Gastrodin can mediate microglia activation through its inhibition of Wnt/β-catenin signaling pathway.

Taken together, it is safe to conclude that Gastrodin can provide protective effects against brain damage caused by the neuroinflammatory response. However, it remains to be ascertained whether Gastrodin would exert its effects directly on the signaling regulatory proteins or otherwise in activated microglia. There remains a lacuna of knowledge whether a specific or cognate "Gastrodin receptor" exists in microglia. This remains a conundrum given the fact that microglial activation is regulated by multiple signaling pathways (Cao et al., 2017) as well as a myriad of microRNAs (Karthikeyan et al., 2018) which may serve as the targets of Gastrodin.

### *Scutellarin*

Scutellarin, a flavone glucuronide (4, 5, 6-trihydroxyflavone-7-glucuronide), is the major active component extracted from the traditional Chinese medicine plant *Erigeron breviscapus* (Vant.) Hand - Mazz (Zhang et al., 2009). It has been widely used in different pathological conditions in China, in particular cerebrovascular diseases (Liu et al., 2005; Wang et al., 2011b). Scutellarin is mainly absorbed in the intestine through the hydrolysis by β-glucuronidases of the intestinal microflora (Gao et al., 2011; Wang et al., 2011c). It is described to be absorbed in the form of scutellarein which can diffuse through the intestinal epithelial membrane effectively.

Accumulating evidence in the past three decades has shown the pharmacological effects of scutellarin in many pathological conditions, especially in regard to its therapeutic effects in cerebral ischemic stroke, neurodegeneration, coronary heart disease, and diabetic complications in animal models (Lin et al., 2007b; Guo et al., 2011; Li et al., 2015; Long et al., 2015). Scutellarin and breviscapine are commonly used in the clinical treatment of angina pectoris, myocardial infarction, hypertension, arrhythmia, hyperlipidemia, chronic heart failure and other coronary heart diseases or ischemic heart diseases. Studies have shown that

scutellarin can improve endothelium dysfunction of coronary artery against myocardial ischemia reperfusion injury (Li et al., 2015). Moreover, scutellarin increased the serum superoxide dismutase, NO and high-density lipoprotein cholesterol, while it decreased the serum malondialdehyde, triglycerides and total cholesterol in a rat atherosclerotic model induced by high fatty diet combined with immunologic injury. These findings indicate that scutellarin has antioxidant activities and can improve dyslipidemia and suppress the aggravation of aorta atherosclerosis in rats (Gao et al., 2011). In a type II diabetes rat model, scutellarin treatment significantly inhibited hyperglycemia-induced testicular cell apoptosis and morphologic impairments (Long et al., 2015). During the development of diabetic retinopathy, scutellarin alleviated BBB oxidative stress injury elicited by activated microglia (Mei et al., 2019).

In neurodegenerative diseases such as in amyotrophic lateral sclerosis mouse model induced by cuprizone-a copper chelator, a demyelination inducer, it was found that scutellarin can improve motor dysfunction, alleviate behavioral deficits, inhibit apoptosis of lateral ventricles subventricular zone neuro stem cells and other deteriorating parameters (Wang et al., 2016). In an AD rat model, scutellarin decreased lipid peroxidation, and free radicals, as well as pathological alterations, such as nuclear shrinking, cellular edema, and the irregular arrangement of the pyramidal layer in the hippocampal CA (1) region. (Guo et al., 2011, 2013). It also ameliorated learning and memory deficit via suppressing Aβ formation and microglia activation in rats with chronic cerebral hypoperfusion (Shin et al., 2018).

In addition to the protective effects on neurodegeneration, scutellarin also exerts protection against cerebrovascular ischemic injury. Several studies have demonstrated the efficacy of scutellarin in protecting against cerebral ischemic injury in rats induced by MCAO. In MCAO, pretreatment of the rats with scutellarin by intragastric administration or intravenous administration significantly reduced the infarction volume and ameliorated neurologic deficits and cell apoptosis induced by ischemia with or without reperfusion. Scutellarin pretreatment decreased BBB permeability in ischemia injury (Lin et al., 2007b; Wang et al., 2016). Both scutellarin and scutellarein effectively attenuated neural cell damage, cerebral water content and a number of biochemical markers after oral administration in bilateral common carotid artery occlusion, a global ischemia animal model; however scutellarein has been found to show a better protective effect than scutellarin (Tang et al., 2014). In a separate study, scutellarin was more effective than edaravone in reducing the infarct area after ipsilateral MCAO. In this connection, the combination of scutellarin and edaravone produced a synergistic anti-ischemic to the effect that one plus one is greater than two (Yuan et al., 2014).

An I/R injury is the tissue damage caused when blood supply returns to the tissue after a period of ischemia. This process of reperfusion can itself induce cell and tissue injury, known as reperfusion injury, for which there is no effective therapy (Hausenloy and Yellon, 2013). The pathophysiology of I/R injury is complex (Kalogeris et al., 2012) and is associated with the decrease of intracellular ATP level, pH value, anaerobic metabolism, and lactate accumulation induced by hypoxia. Upon reperfusion, the oxygen level is restored, causing a massive generation of reactive oxygen species (ROS), infiltration of inflammatory cells, and endothelial and vascular dysfunction among others. All this would lead to cell death. Scutellarin has been shown to be effective or beneficial for the treatment of ischemic stroke and myocardial injury, attributable to its anti-inflammatory, anti-oxidant and anti-apoptosis activities.

CNS injury is characterized by immune cell activation and production of inflammatory mediators. In this connection, microglial cells which are considered to be the resident innate immune cells are responsible for the immune surveillance in the CNS (Aguzzi et al., 2013). Upon exposure to stimulus, microglia responded swiftly to mediate many of the pathologic processes, and release excessive amounts of proinflammatory cytokines and cytotoxic agents (Yuan et al., 2016). Scutellarin inhibits the microglia-mediated inflammatory response. In a separate study, scutellarin was shown to attenuate LPS-induced production of proinflammatory mediators, including NO, TNF-α, IL-1β and reactive oxygen species (ROS) in cultured primary rat microglia or the BV-2 mouse microglial cell line (Wang et al., 2011a), as well as in activated microglia of rats subjected to MCAO (Yuan et al., 2014). Furthermore, scutellarin enhanced the expression of nerve growth factors, brain-derived neurotrophic factor, and the glial cell-derived neurotrophic factor in astrocytes under hypoxia/reoxygenation (Chai et al., 2013). Scutellarin increased the ability of adhesion, inhibited migration *in vitro* and *in vivo* and promoted the formation of flattened and microspike projections by promoting the reorganization and stabilization of cytoskeletal dynamics in activated microglial cells (Yuan et al., 2015). In MCAO rats, scutellarin increased the expression of the glial fibrillary acidic protein and nestin as well as proinflammatory mediators in reactive astrocytes during ischemic injury. Scutellarin promotes astrogliosis via activated microglia, which suggests a cross-talk between microglia and astrocytes during ischemic injury (Fang et al., 2015).

The pathways whereby scutellarin can inhibit microglia-mediated neuroinflammation during I/R injury remain to be fully clarified. It is well documented that scutellarin can effectively decrease the expression of NF-ϰB, a key transcription factor that regulates the proinflammatory gene expression such as TNF-α, IL-1β, IL-6, IL-8, iNOS, and COX-2 (D'Acquisto et al., 2002; Wang et al., 2011b; Chen et al., 2013). We have found that scutellarin can inhibit the expression of NF-ϰB in activated BV-2 microglia and activated microglia in

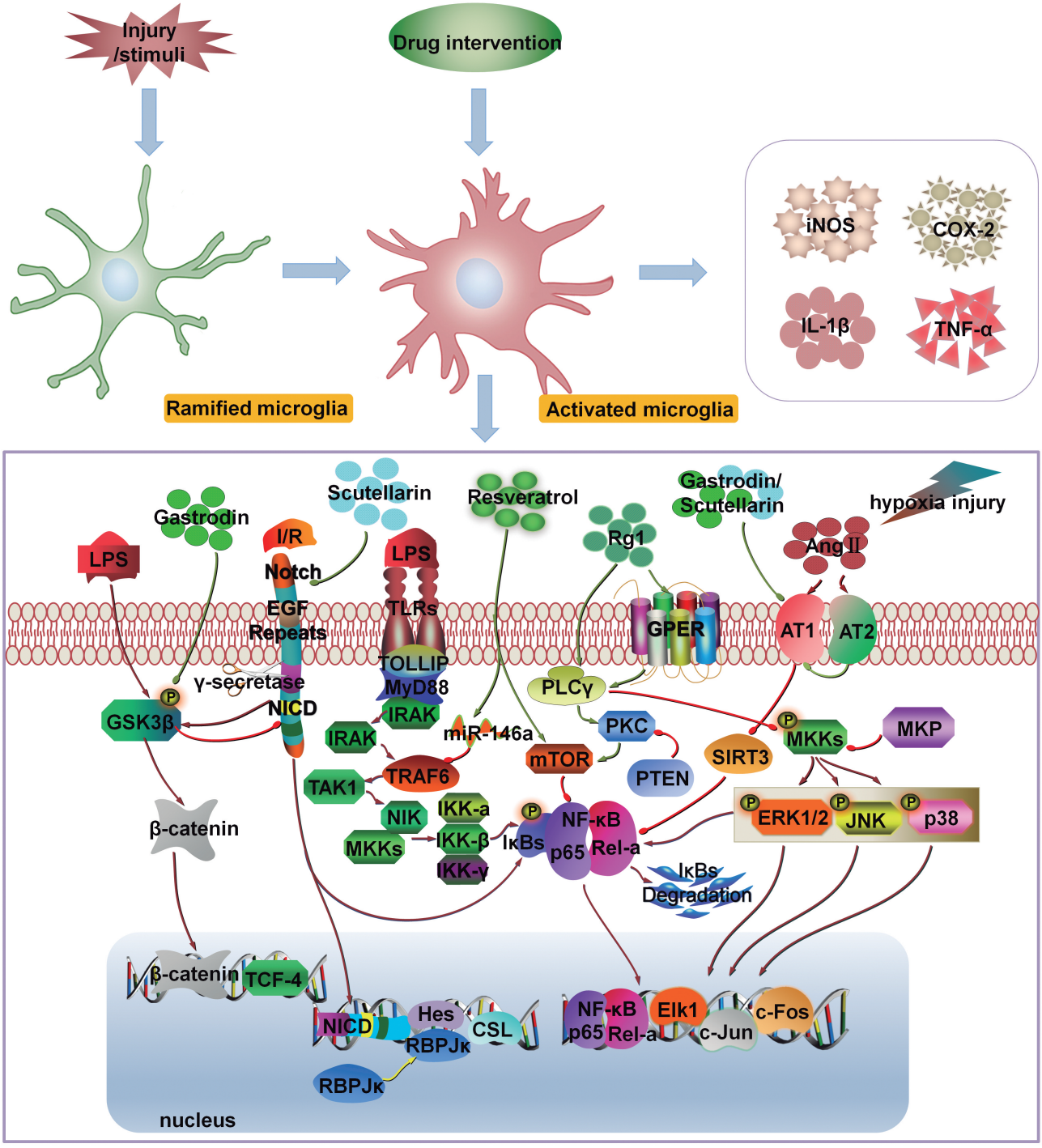


**Fig. 1.** A schematic diagram depicting the regulation of microglia activation by different signaling pathways and natural compound intervention. When challenged by various stimuli, including brain injuries, microglia are activated and undergo swift morphological transformation from the ramified- into the amoeboid phenotype. The activated microglia, preferentially the amoeboid phenotype, secrete an array of proinflammatory mediators such as IL-1β and TNF-α. Multiple signaling pathways are involved in regulating microglia activation and in some instances extend over a prolonged duration. Of the many signaling pathways driving the process and which have been featured prominently include the Notch-1, PLC-γ, mTOR, MAPK and NF-κB signaling pathways. Several natural compounds, including some well-known herbal extracts that possess anti-inflammatory properties, have shown great potential for amelioration of microglia activation and, hence, are neuroprotective. Among the various natural compounds, gastrodin has been shown unequivocally to decrease the microglia activation induced by LPS and hypoxia injury by targeting the GSK-3β and the RAS system. Scutellarin is highly potent in its anti-inflammatory effects in activated microglia, as demonstrated in I/R injury through Notch-1 signaling. Resveratrol not only regulates the mTOR signal but also acts on the miR-146. Ginsenoside Rg1 plays a central role in regulating microglia activation through PLC-γ and GPER. All in all, therefore, the above-mentioned compounds can suppress microglia activation and mitigate the microglia mediated neuroinflammation that is implicated in different neuropathological conditions. The specific target proteins of the respective compounds, however, remain to be identified.

MCAO rats (Yuan et al., 2015). Recently, it has been reported that scutellarin exerts its anti inflammatory effects in activated microglia/brain macrophages in cerebral ischemia and in activated BV-2 microglia through regulation of the MAPKs signaling pathway. In this regard, scutellarin markedly attenuated the phosphorylation of p38 and JNK in activated microglia/brain macrophages in MCAO rats. As opposed to this, the phosphorylation of ERK1/2 was significantly increased by scutellarin. The results suggest that scutellarin down-regulates the expression of proinflammatory mediators in activated microglia/brain macrophages by suppressing the phosphorylation of JNK and p38 MAPK. Of note, the anti-inflammatory effect of p38 MAPK inhibitor and scutellarin is comparable. Besides, p38 MAPK activator reverses the effect of scutellarin on inhibition of the JNK, p38 MAPK and the inflammatory process (Chen et al., 2020). Thus, the underlying mechanism of scutellarin in regulating microglia activation remains complex as it may exert its effects on multiple signaling pathways or targets.

Remarkably, scutellarin can exert its effects on Notch-1 signaling. To this end, scutellarin has been shown to markedly attenuate the expression of Notch- 1, NICD, recombining binding protein suppressor of hairless and hairy and enhancer of split-1 (Hes-1) in activated BV-2 microglia and activated microglia in MCAO rats. Scutellarin can also decrease NF-κB expression in the same cells (Yuan et al., 2015). These results indicated that scutellarin plays an anti-inflammatory role by targeting the Notch pathway, which lies upstream of NF-κB (Schwarzer et al. 2012).

As discussed above, RAS signaling plays multiple roles in cerebral vascular functions, including neuroinflammation and brain ischemic injury. AutoDock software (version 4.2, http://autodock.scripps.edu/)-based analysis indicates that scutellarin selectivity binds to ACE with high affinity. It has been reported that scutellarin reduces the expression of ACE and AT1R, as well as that of AngII, TNF-α, IL-6, and IL-1β in ischemic brains. These findings indicate that scutellarin can protect the brain from acute ischemic injury via regulation of the ACE/AngII/AT1 axis in activated microglia through inhibition of production of proinflammatory cytokine (Wang et al., 2016).

## Conclusion

It is well documented that microglia-mediated neuroinflammation contributes to the progression of different brain pathologies. In this connection, activated microglia, the key players of the inflammatory process, release excess amounts of pro-inflammatory mediators that can exacerbate the tissue injuries. It is therefore justified to suggest that suppression of microglial activation would be an effective therapeutic strategy to quell neuroinflammation for optimal tissue repair and ultimately restoration of neurological function. In view of this, it is not surprising that great efforts have been undertaken by many in recent years aiming to decipher the molecular mechanisms that drive microglia activation. Among the various signaling pathways that regulate microglia activation as demonstrated in our own studies are NF-κB, mTOR, MAPKs, Notch-1, RAS pathways etc. and as discussed in this review and depicted in Fig 1. Undoubtedly microglia activation is a complex process; indeed, its complexity and the interlinking of multiple signaling pathways involved have greatly hampered progress to design an optimal therapeutic strategy for mitigation of microglia mediated neuroinflammation. Search for therapeutic agents from natural compounds, notably some traditional Chinese herbal medicines, has become a promising direction for treatment of a wide range of neuropathological conditions. The main advantage of herbal and natural products is that they are relatively safe, inexpensive, and readily available. Natural compounds or herbal extracts such as resveratrol, gastrodin, scutellarin and ginsenoside Rg1 have been extensively studied in recent years. All experimental evidence seems to converge that they are endowed with anti-inflammatory and anti-oxidant properties. While extensive work has been done on gastrodin as well as the other three common herbal extracts as discussed in this review on their anti-inflammatory and antioxidant effects in activated microglia, the underlying molecular mechanisms have remained obscure. Already we have shown that they can decrease the NF-κB, Notch-1, RAS and MAPKs pathways, but the underlying mechanism by which they act on suppression of microglial activation remains uncertain. The target proteins of the respective compounds remain elusive. This is compounded by many recent findings on regulation of microglia activation by additional epigenetic mechanisms. For instance, resveratrol targets miR-146 against the activation of microglia *in vitro*. It also remains to be ascertained whether the therapeutic effects as shown by the experimental settings may be applied to humans and in a clinical setting. A recent description of the so-called M1/M2 microglia phenotypes has added a new perspective on the roles of activated microglia in brain pathologies (Yuan et al., 2019). Whether the natural compounds here reviewed would help M2 polarization, deemed to be neuroprotective by many, should be the scope of future study.

*Acknowledgements.* This work was supported in part by National Science Foundation of China (No. 31760292 and 81460210), the Department project of Science and Technology of Yunnan Province (No. 2017FA035 and 2019ZF011-2) and the Department project of Education of Yunnan Province (No. K13225031).

## References

Aguzzi A., Barres B.A. and Bennett M.L. (2013). Microglia: scapegoat, saboteur, or something else? Science 339, 156-161.

Ahn B.H., Kim H.S., Song S., Lee I.H., Liu J., Vassilopoulos A., Deng C.X. and Finkel T. (2008). A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc. Natl. Acad. Sci. USA 105, 14447-14452.

Ajmone-Cat M.A., D'Urso M.C., Blasio G., Brignone M.S., Simone R.D. and Minghetti L. (2016). Glycogen synthase kinase 3 is part of the molecular machinery regulating the adaptive response to LPS stimulation in microglial cells. Brain Behav. Immun. 55, 225-235.

Alliot F., Godin I. and Pessac B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res. Dev. Brain Res. 117, 145-152.

Anekonda T.S. (2006) Resveratrol-a boon for treating Alzheimer's disease?. Brain Res. Rev. 52, 316-326.

Ang H.L. and Tergaonkar V. (2007). Notch and NF-κB signaling pathways: Do they collaborate in normal vertebrate brain development and function? BioEssays 29, 1039-1047.

Aronica E., Fluiter K., Iyer A., Zurolo E., Vreijling J., Vliet E.A., Baayen J.C. and Gorter J.A. (2010). Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. Eur. J. Neurosci. 31, 1100-1107.

Arumugam T.V., Chan S.L., Jo D.G., Yilmaz G., Tang S.C., Cheng A., Gleichmann M., Okun E., Dixit V.D., Chigurupati S., Mughal M.R., Ouyang X., Miele L., Magnus T., Poosala S., Granger D.N. and Mattson M.P. (2006). Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. Nat. Med. 12, 621-623.

Athar M., Back J.H., Tang X., Kim K.H., Kopelovich L., Bickers D.R. and Kim A.L. (2007). Resveratrol: a review of preclinical studies for human cancer prevention. Toxicol. Appl. Pharmacol. 224, 274-283.

Bae E.A., Kim E.J., Park J.S., Kim H.S., Ryu J.H. and Kim D.H. (2006). Ginsenosides Rg3 and Rh2 inhibit the activation of AP-1 and protein kinase A pathway in lipopolysaccharide/interferon-gamma-stimulated BV-2 microglial cells. Planta Med. 72, 627-633.

Baek S.H., Bae O.N. and Park J.H. (2012). Recent methodology in ginseng analysis. J. Ginseng Res. 36, 119-134.

Baker N., O'Meara S.J., Scannell M., Maderna P. and Godson C. (2009). Lipoxin A4: anti-inflammatory and anti-angiogenic impact on endothelial cells. J. Immunol. 182, 3819-3826.

Baur J.A. and Sinclair D.A. (2006). Therapeutic potential of resveratrol: the *in vivo* evidence. Nat. Rev. Drug. Discov. 5, 493-506.

Bean L.A., Ianov L. and Foster T.C. (2014). Estrogen receptors, the hippocampus, and memory. Neuroscientist 20, 534-545.

Bell E.L. and Guarente L. (2011). The Sirt3 divining rod points to oxidative stress. Mol. Cell. 42, 561-568.

Bell E.L., Emerling B.M., Ricoult S.J. and Guarente L. (2011). Sirt3 suppresses hypoxia inducible factor 1alpha and tumor growth by inhibiting mitochondrial ROS production. Oncogene 30, 2986-2996.

Benicky J., Sanchez-Lemus E., Pavel J. and Saavedra J.M. (2009). Anti-inflammatory effects of angiotensin receptor blockers in the brain and the periphery. Cell. Mol. Neurobiol. 29, 781-792.

Beurel E. and Jope R.S. (2009). Lipopolysaccharide-induced interleukin-6 production is controlled by glycogen synthase kinase-3 and STAT3 in the brain. J. Neuroinflammation 6, 9-9.

Beurel E., Michalek S.M. and Jope R.S. (2010). Innate and adaptive immune responses regulated by glycogen synthase kinase-3 (GSK3). Trends Immunol. 31, 24-31.

Bi X.L., Yang J.Y., Dong Y.X., Wang J.M., Cui Y.H., Ikeshima T., Zhao Y.Q. and Wu C.F. (2005). Resveratrol inhibits nitric oxide and TNF-alpha production by lipopolysaccharide-activated microglia. Int. Immunopharmacol. 5, 185-193.

Bray S.J. (2006). Notch signalling: a simple pathway becomes complex. Nat. Rev. Mol. Cell. Biol. 7, 678-689.

Brouns R. and Deyn P.P. (2009). The complexity of neurobiological processes in acute ischemic stroke. Clin. Neurol. Neurosurg. 111, 483-495.

Cai Z., Hou S., Li Y., Zhao B., Yang Z., Xu S. and Pu J. (2008). Effect of borneol on the distribution of gastrodin to the brain in mice via oral administration. J. Drug Target 16, 178-184.

Cai B., Seong K.J., Bae S.W., Chun C., Kim W.J. and Jung J.Y. (2018). A synthetic diosgenin primary amine derivative attenuates LPS-stimulated inflammation via inhibition of NF-κB and JNK MAPK signaling in microglial BV2 cells. Int. Immunopharmacol. 61, 204-214.

Cai B., Seong K.J., Bae S.W., Kook M.S., Chun C., Lee J.H., Choi W.S., Jung J.Y. and Kim W.J. (2019). Water-soluble arginyl-diosgenin analog attenuates hippocampal neurogenesis impairment through blocking microglial activation underlying NF-κB and JNK MAPK signaling in adult mice challenged by LPS. Mol. Neurobiol. 59, 6218-6238.

Canon E., Cosgaya J.M., Scsucova S. and Aranda A. (2004). Rapid effects of retinoic acid on CREB and ERK phosphorylation in neuronal cells. Mol. Biol. Cell 15, 5583-5592.

Cao Q., Lu J., Kaur C., Sivakumar V., Li F., Cheah P.S., Dheen S.T. and Ling E.A. (2008). Expression of Notch-1 receptor and its ligands Jagged-1 and Delta-1 in amoeboid microglia in postnatal rat brain and murine BV-2 cells. Glia 56, 1224-1237.

Cao Q., Li P., Lu J., Dheen S.T., Kaur C and Ling E.A. (2010). Nuclear factor-κB/p65 responds to changes in the Notch signaling pathway in murine BV-2 cells and in amoeboid microglia in postnatal rats treated with the gamma-secretase complex blocker DAPT. J. Neurosci. Res. 88, 2701-2714.

Cao Q., Kaur C., Wu C.Y., Lu J. and Ling E.A. (2011). Nuclear factor-kappa beta regulates Notch signaling in production of proinflammatory cytokines and nitric oxide in murine BV-2 microglial cells. Neuroscience 192, 140-154.

Cao Q., Karthikeyan A., Dheen S.T., Kaur C. and Ling E.A. (2017). Production of proinflammatory mediators in activated microglia is synergistically regulated by Notch-1, glycogen synthase kinase (GSK-3β) and NF-κB/p65 signalling. PLoS One 12, e0186764.

Capiralla H., Vingtdeux V., Zhao H., Sankowski R., Al-Abed Y., Davies P. and Marambaud P. (2012). Resveratrol mitigates lipopolysaccharide- and Aβ-mediated microglial inflammation by inhibiting the TLR4/NF-κB/STAT signaling cascade. J. Neurochem. 120, 461-472.

Chai L., Guo H., Li H., Wang S., Wang YL., Shi F., Hu L.M., Liu Y. and Adah D. (2013). Scutellarin and caffeic acid ester fraction, active components of Dengzhanxixin injection, upregulate neurotrophins synthesis and release in hypoxia/reoxygenation rat astrocytes. J. Ethnopharmacol. 150, 100-107.

Chen X., Shi X., Zhang X., Lei H., Long S., Pei Z. and Huang R. (2013). Scutellarin attenuates hypertension-induced expression of brain toll-like receptor 4/nuclear factor kappa B. Mediators. Inflamm. 2013, 432623.

Chen H.L., Jia W.J., Li H.E., Han H., Li F., Zhang X.L., Li J.J., Yuan Y. and Wu C.Y. (2020). Scutellarin exerts anti-infammatory effects in activated microglia/brain macrophage in cerebral ischemia and in activated BV-2 microglia through regulation of MAPKs signaling pathway. Neuromolecular. Med. 22, 264-277.

Cui Y., Wang Y., Zhao D., Feng X., Zhang L. and Liu C. (2018). Loganin prevents BV-2 microglia cells from Aβ 1-42 -induced inflammation via regulating TLR4/TRAF6/NF-κB axis. Cell. Biol. Int. 42, 1632-1642.

D'Acquisto F., May M.J. and Ghosh S. (2002). Inhibition of nuclear factor kappa B (NF-B): an emerging theme in anti-inflammatory therapies. Mol. Interv. 2, 22-35.

Dai J.N., Zong Y., Zhong L.M., Li Y.M., Zhang W., Bian L.G., Ai Q.L., Liu Y.D., Sun J. and Lu D. (2011). Gastrodin inhibits expression of inducible NO synthase, cyclooxygenase-2 and proinflammatory cytokines in cultured LPS-stimulated microglia via MAPK pathways. PLoS One 6, e21891.

Danielyan L., Lourhmati A., Verleysdonk S., Kabisch D., Proksch B., Thiess U., Umbreen S., Schmidt B. and Gleiter C.H. (2007). Angiotensin receptor type 1 blockade in astroglia decreases hypoxia-induced cell damage and TNF alpha release. Neurochem. Res. 32, 1489-1498.

Dheen S.T, Kaur C. and Ling E.A. (2007). Microglial activation and its implications in the brain diseases. Curr. Med. Chem. 14, 1189-1197.

Doble B.W. and Woodgett J.R. (2003). GSK-3: tricks of the trade for a multi-tasking kinase. J. Cell. Sci. 16, 1175-1186.

Dou Y., Tian X., Zhang J., Wang Z. and Chen G. (2018). Roles of TRAF6 in central nervous system. Curr. Neuropharmacol. 16, 1306-1313.

Du L., Zhang Y., Chen Y., Zhu J., Yang Y. and Zhang H.L. (2017). Role of microglia in neurological disorders and their potentials as a therapeutic target. Mol. Neurobiol. 54, 7567-7584.

Erlich S., Alexandrovich A., Shohami E. and Pinkas-Kramarski R. (2007). Rapamycin is a neuroprotective treatment for traumatic brain injury. Neurobiol. Dis. 26, 86-93.

Fan C., Song Q., Wang P., Li Y., Yang M. and Yu S.Y. (2018). Neuroprotective effects of ginsenoside-Rg1 against depression-like behaviors via suppressing glial activation, synaptic deficits, and neuronal apoptosis in rats. Front. Immunol. 9, 2889.

Fang F., Chen X., Huang T., Lue L.F., Luddy, J.S. and Yan S.S. (2012). Multi-faced neuroprotective effects of Ginsenoside Rg1 in an Alzheimer mouse model. Biochim. Biophys. Acta 1822, 286-292.

Fang M., Yuan Y., Rangarajan P., Lu J., Wu Y., Wang H., Wu C. and Ling E.A. (2015). Scutellarin regulates microglia-mediated TNC1 astrocytic reaction and astrogliosis in cerebral ischemia in the adult rats. BMC Neurosci. 16, 84-84.

Fang H., Zhang J.C., Yang M., Li H.F., Zhang J.P., Zhang F.X., Wang Q.Y., Wang R.R. and Liu J. (2016). Perfusion of gastrodin in abdominal aorta for alleviating spinal cord ischemia reperfusion injury. Asian Pac. J. Trop. Med. 9, 688-693.

Fu G., Chen Y., Yu M.; Podd A., Schuman J., He Y., Di L., Yassai M., Haribhai D., North P.E., Gorski J., Williams C.B., Wang D. and Wen R. (2010). Phospholipase Cγ1 is essential for T cell development, activation, and tolerance. J. Exp. Med. 207, 309-318.

Gao C., Chen X. and Zhong D. (2011). Absorption and disposition of scutellarin in rats: a pharmacokinetic explanation for the high exposure of its isomeric metabolite. Drug Metab. Dispos. 39, 2034-2044.

Gao X.Q., Du Z.R., Yuan L.J., Zhang W.D., Chen L., Teng J.J., Wong M.S., Xie J.X. and Chen W.F. (2019). Ginsenoside Rg1 exerts anti-inflammatory effects via g protein-coupled estrogen receptor in lipopolysaccharide-induced microglia activation. Front. Neurosci. 13, 1168-1168.

Gatson J.W., Liu M.M., Abdelfattah K., Wigginton J.G., Smith S., Wolf S. and Minei J.P. (2013). Resveratrol decreases inflammation in the brain of mice with mild traumatic brain injury. J. Trauma Acute Care Surg. 74, 470-474.

Gaudet A.D., Fonken L.K., Watkins L.R., Nelson R.J. and Popovich P.G. (2018). MicroRNAs: roles in regulating neuroinflammation. Neuroscientist 24, 221-245.

Ge Y.T., Zhong A.Q., Xu G.F. and Lu Y. (2019). Resveratrol protects BV2 mouse microglial cells against LPS-induced inflammatory injury by altering the miR-146a-5p/TRAF6/NF-κB axis. Immunopharmacol. Immunotoxicol. 41, 549-557.

Gendron L., Payet M.D. and Gallo-Payet N. (2003). The angiotensin type 2 receptor of angiotensin II and neuronal differentiation: from observations to mechanisms. J. Mol. Endocrinol. 31, 359-372.

Gentle M.E., Rose A., Bugeon L. and Dallman M.J. (2012). Noncanonical Notch signalling modulates cytokine responses of dendritic cells to inflammatory stimuli. J. Immunol. 189, 1274-1284.

Gentleman S.M., Leclercq P.D., Moyes L., Graham D.I., Smith C., Griffin W.S. and Nicoll J.A. (2004). Long-term intracerebral inflammatory response after traumatic brain injury. Forensic Sci. Int. 146, 97-104.

Ghosh A., Roy A., Liu X., Kordower J.H., Mufson E.J., Hartley D.M., Ghosh S., Mosley R.L., Gendelman H.E. and Pahan K. (2007). Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. Proc. Natl. Acad. Sci. USA 104, 18754-18759.

Ginhoux F., Greter M., Leboeuf M., Nandi S., See P., Gokhan S., Mehler M.F., Conway S.J., Ng L.G., Stanley E.R., Samokhvalov I.M. and Merad M. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330, 841-845.

Gocmez S.S., Gacar N., Utkan T., Gacar G., Scarpace P.J. and Tumer N. (2016). Protective effects of resveratrol on aging-induced cognitive impairment in rats. Neurobiol. Learn. Mem. 131, 131-136.

Greter M. and Merad M. (2013). Regulation of microglia development and homeostasis. Glia 61, 121-127.

Griendling K.K., Minieri C.A., Ollerenshaw J.D. and Alexander R.W. (1994). Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ. Res. 74, 1141-1148.

Grimes C.A. and Jope R.S. (2001). The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Prog. Neurobiol. 65, 391-426.

Guan J., Yang B., Fan Y. and Zhang J. (2017). GPER agonist G1 attenuates neuroinflammation and dopaminergic neurodegeneration in Parkinson disease. Neuroimmunomodulation 24, 60-66.

Guimarães M.R., Leite F.R., Spolidorio L.C., Kirkwood K.L. and Jr C.R. (2013). Curcumin abrogates LPS-induced pro-inflammatory cytokines in RAW 264.7 macrophages. Evidence for novel mechanisms involving SOCS-1, -3 and p38 MAPK. Arch. Oral. Biol. 58, 1309-1317.

Guimond M.O. and Gallo-Payet N. (2012). How does angiotensin AT(2) receptor activation help neuronal differentiation and improve neuronal pathological situations? Front. Endocrinol. (Lausanne) 3, 164-164.

Guo L.L., Guan Z.Z. and Wang Y.L. (2011). Scutellarin protects against Aβ-induced learning and memory deficits in rats: involvement of nicotinic acetylcholine receptors and cholinesterase. Acta Pharmacol. Sin. 32, 1446-1453.

Guo L.L., Guan Z.Z., Huang Y., Wang Y.L. and Shi J.S. (2013). The neurotoxicity of beta-amyloid peptide toward rat brain is associated with enhanced oxidative stress, inflammation and apoptosis, all of which can be attenuated by scutellarin. Exp. Toxicol. Pathol. 65,

579-584.

Ha J.H., Shin S.M., Lee S.K., Kim J.S., Shin U.S., Huh K., Kim J.A., Yong C.S., Lee N.J. and Lee D.U. (2001). *In vitro* effects of hydroxybenzaldehydes from *Gastrodia elata* and their analogues on GABAergic neurotransmission, and a structure-activity correlation. Planta Med. 67, 877-880.

Haddadi R., Poursina M., Zeraati F. and Nadi F. (2018). Gastrodin microinjection suppresses 6-OHDA-induced motor impairments in parkinsonian rats: insights into oxidative balance and microglial activation in SNc. Infammopharmacology 26, 1305-1316.

Halleskog C., Mulder J., Dahlström J., Mackie K., Hortobágyi T., Tanila H., Kumar Puli L., Färber K., Harkany T. and Schulte G. (2011). WNT signaling in activated microglia is proinflammatory. Glia 59, 119-131.

Hasegawa H. (2004). Proof of the mysterious efficacy of ginseng: basic and clinical trials: metabolic activation of ginsenoside: deglycosylation by intestinal bacteria and esterification with fatty acid. J. Pharmacol. Sci. 95, 153-157.

Hasegawa H. and Uchiyama M. (1998). Antimetastatic efficacy of orally administered ginsenoside Rb1 in dependence on intestinal bacterial hydrolyzing potential and significance of treatment with an active bacterial metabolite. Planta Med. 64, 696-700.

Hasegawa H., Sung J.H. and Benno Y. (1997). Role of human intestinal Prevotella oris in hydrolyzing ginseng saponins. Planta Med. 63, 436-440.

Hausenloy D.J. and Yellon D.M. (2013). Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J. Clin. Invest. 123, 92-100.

Hayashi H., Campenot R.B., Vance D.E. and Vance J.E. (2009). Protection of neurons from apoptosis by apolipoprotein E-containing lipoproteins does not require lipoprotein uptake and involves activation of phospholipase C gamma1 and inhibition of calcineurin. J. Biol. Chem. 284, 29605-29613.

He Q., Sun J., Wang Q., Wang W. and He B. (2014). Neuroprotective effects of ginsenoside Rg1 against oxygen-glucose deprivation in cultured hippocampal neurons. J. Chin. Med. Assoc. 77, 142-149.

He X., Zheng Y., Liu S., Shi S., Liu Y., He Y., Zhang C. and Zhou X. (2018). MiR-146a protects small intestine against ischemia/reperfusion injury by down-regulating TLR4/TRAF6/NF-κB pathway. J. Cell. Physiol. 233, 2476-2488.

Heng Y., Zhang Q.S., Mu Z., Hu J.F., Yuan Y.H. and Chen N.H. (2016). Ginsenoside Rg1 attenuates motor impairment and neuroinflammation in the MPTP-probenecid-induced parkinsonism mouse model by targeting α-synuclein abnormalities in the substantia nigra. Toxicol. Lett. 243, 7-21.

Hou J.G., Xue J.J., Sun M.Q., Wang C.Y., Liu L., Zhang D.L., Lee M.R., Gu L.J., Wang C.L., Wang Y.B., Zheng Y., Li W. and Sung C.K. (2012). Highly selective microbial transformation of major ginsenoside Rb1 to gypenoside LXXV by Esteya vermicola CNU120806. J. Appl. Microbiol. 113, 807-814.

Hsieh C.L., Lin J.J., Chiang S.Y., Su S.Y., Tang N.Y., Lin G.G., Lin I.H., Liu C.H., Hsiang C.Y., Chen J.C. and Ho T.Y. (2007). Gastrodia elata modulated activator protein 1 via c-Jun N-terminal kinase signaling pathway in kainic acid-induced epilepsy in rats. J. Ethnopharmacol. 109, 241-247.

Huang T.C., Lu K.T., Wo Y.Y., Wu Y.J. and Yang Y.L. (2011). Resveratrol protects rats from Aβ-induced neurotoxicity by the reduction of iNOS expression and lipid peroxidation. PLoS One 6, e29102.

Imler T.J. Jr and Petro T.M. (2009). Decreased severity of experimental autoimmune encephalomyelitis during resveratrol administration is associated with increased IL-17+IL-10+ T cells, CD4(-) IFN-gamma+ cells, and decreased macrophage IL-6 expression. Int. Immunopharmacol. 9, 134-143.

Jacobs A.H. and Tavitian B. (2012). Noninvasive molecular imaging of neuroinflammation. J. Cereb. Blood. Flow. Metab. 32, 1393-1415.

Jadhav S.P., Kamath S.P., Choolani M., Lu J. and Dheen S.T. (2014). microRNA-200b modulates microglia-mediated neuroinflammation via the cJun/MAPK pathway. J. Neurochem. 130, 388-401.

Ji Q.S., Winnier G.E., Niswender K.D., Horstman D., Wisdom R., Magnuson M.A. and Carpenter G. (1997). Essential role of the tyrosine kinase substrate phospholipase C-gamma1 in mammalian growth and development. Proc. Natl. Acad. Sci. USA 94, 2999-3003.

Jones E.S., Vinh A., McCarthy C.A., Gaspari T.A. and Widdop R.E. (2008). AT2 receptors: functional relevance in cardiovascular disease. Pharmacol. Ther. 120, 292-316.

Juknat A., Gao F., Coppola G., Vogel Z. and Kozela E. (2019). miRNA expression profiles and molecular networks in resting and LPS-activated BV-2 microglia effect of cannabinoids. PLoS One 14, e0212039.

Jung I.H., Lee J.H., Hyun Y.J. and Kim D.H. (2012). Metabolism of ginsenoside Rb1 by human intestinal microflora and cloning of its metabolizing β-D-glucosidase from Bifidobacterium longum H-1. Biol. Pharm. Bull. 35, 573-581.

Jung J.W., Yoon B.H., Oh H.R., Ahn J.H., Kim S.Y., Park S.Y. and Ryu J.H. (2006). Anxiolytic-like effects of *Gastrodia elata* and its phenolic constituents in mice. Biol. Pharm. Bull. 29, 261-265.

Jung K.H., Chu K., Lee S.T., Kim S.J., Song E.C., Kim E.H., Park D.K., Sinn D.I., Kim J.M., Kim M. and Roh J.K. (2007). Blockade of AT1 receptor reduces apoptosis, inflammation, and oxidative stress in normotensive rats with intracerebral hemorrhage. J. Pharmacol. Exp. Ther. 322, 1051-1058.

Kalogeris T., Baines C.P., Krenz M. and Korthuis R.J. (2012). Cell biology of ischemia/reperfusion injury. Int. Rev. Cell. Mol. Biol. 298, 229-317.

Karin M. and Gallagher E. (2009). TNFR signaling: ubiquitin-conjugated TRAFfic signals control stop-and-go for MAPK signaling complexes. Immunol. Rev. 228, 225-240.

Karthikeyan A., Gupta N., Tang C., Mallilankaraman K., Silambarasan M., Shi M., Lu L., Ang B.T., Ling E.A. and Dheen S.T., (2018). Microglial SMAD4 regulated by microRNA-146a promotes migration of microglia which support tumor progression in a glioma environment. Oncotarget 9, 24950-24969.

Karthikeyan A., Patnala R., Jadhav S.P., Ling E.A. and Dheen S.T. (2016). MicroRNAs: key players in microglia and astrocyte mediated inflammation in CNS pathologies. Curr. Med. Chem. 23, 3528-3546.

Karuppagounder S.S., Pinto J.T., Xu H., Chen H.L., Beal M.F. and Gibson G.E. (2009). Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. Neurochem. Int. 54, 111-118.

Kawano T., Yamanaka D., Aoyama B., Tateiwa H., Shigematsu-Locatelli M., Nishigaki A., Iwata H., Locatelli F.M. and Yokoyama M. (2018). Involvement of acute neuroinflammation in postoperative delirium-like cognitive deficits in rats. J. Anesth. 32, 506-517.

Keum Y.S., Han S.S., Chun K.S., Park K.K., Park J.H., Lee S.K. and Surh Y.J. (2003). Inhibitory effects of the ginsenoside Rg3 on phorbol ester-induced cyclooxygenase-2 expression, NF-kappaB activation and tumor promotion. Mutat. Res. 523-524, 75-85.

Kiefer D. and Pantuso T. (2003). Panax ginseng. Am. Fam. Physician. 68, 1539-1542.

Kim B.W., Koppula S., Kim J.W., Lim H.W., Hwang J.W., Kim I.S., Park P.J. and Choi D.K. (2012). Modulation of LPS stimulated neuroinflammation in BV-2 microglia by *Gastrodia elata*: 4-hydroxybenzyl alcohol is the bioactive candidate. J. Ethnopharmacol. 139, 549-557.

Kim E.K. and Choi E.J. (2010). Pathological roles of MAPK signaling pathways in human diseases. Biochim. Biophys. Acta 1802, 396-405.

Kincaid B. and Bossy-Wetzel E. (2013). Forever young: SIRT3 a shield against mitochondrial meltdown, aging, and neurodegeneration. Front. Aging Neurosci. 5, 48-48.

Koistinaho J., Malm T. and Goldsteins G. (2011). Glycogen synthase kinase-3β: a mediator of inflammation in Alzheimer's disease? Int. J. Alzheimers. Dis. 129753-129753.

Kubota K., Nakano M., Kobayashi E., Mizue Y., Chikenji T., Otani M., Nagaishi K. and Fujimiya M. (2018). An enriched environment prevents diabetes-induced cognitive impairment in rats by enhancing exosomal miR-146a secretion from endogenous bone marrow-derived mesenchymal stem cells. PLoS One 13, e0204252.

Labandeira-Garcia J.L., Rodriguez-Perez A.I., Garrido-Gil P., Rodriguez-Pallares J., Lanciego J.L. and Guerra M.J. (2017). Brain renin-angiotensin system and microglial polarization: implications for aging and neurodegeneration. Front. Aging Neurosci. 9, 129-129.

Lambert K.A., Roff A.N., Panganiban R.P., Douglas S. and Ishmael F.T. (2018). MicroRNA-146a is induced by inflammatory stimuli in airway epithelial cells and augments the anti-inflammatory effects of glucocorticoids. PLoS One 13, e0205434.

Lawrence T., Gilroy D.W., Colville-Nash P.R. and Willoughby D.A. (2001). Possible new role for NF-kappaB in the resolution of inflammation. Nat. Med. 7, 1291-1297.

Lee Y.H., Kim S., Kim J., Kim Y.K., Kim M.J., Ryu S.H. and Suh P. (1999). Overexpression of phospholipase C-gamma1 suppresses UVC-induced apoptosis through inhibition of c-fos accumulation and c-Jun N-terminal kinase activation in PC12 cells. Biochim. Biophys. Acta 1440, 235-243.

Lee K.S., Kim S.R., Park S.J., Lee H.K., Park H.S., Min K.H., Jin S.M. and Lee Y.C. (2006). Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) reduces vascular endothelial growth factor expression in allergen-induced airway inflammation. Mol. Pharmacol. 69, 1829-1839.

Lee S.Y., Kim G.T., Roh S.H., Song J.S., Kim H.J., Hong S.S., Kwon S.W. and Park J.H. (2009). Proteomic analysis of the anti-cancer effect of 20S-ginsenoside Rg3 in human colon cancer cell lines. Biosci. Biotechnol. Biochem. 73, 811-816.

Lee E.J., Ko H.M., Jeong Y.H., Park E.M. and Kim H.S. (2015). β-Lapachone suppresses inflammation by modulating the expression of cytokines and matrix metalloproteinases in activated microglia. J. Neuroinflammation 12, 133-133.

Li Y. and Zhang Z. (2015). Gastrodin improves cognitive dysfunction and decreases oxidative stress in vascular dementia rats induced by chronic ischemia. Int. J. Clin. Exp. Pathol. 8, 14099-14109.

Li X., Massa P.E., Hanidu A., Peet G.W., Aro P., Savitt A., Mische S., Li J. and Marcu K.B. (2002). IKKalpha, IKKbeta, and NEMO/IKKgamma are each required for the NF-kappa B-mediated inflammatory response program. J. Biol. Chem. 277, 45129-45140.

Li X., Zhu W., Roh M.S., Friedman A.B., Rosborough K. and Jope R.S. (2004). *In vivo* regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. Neuropsychopharmacology 29, 1426-1431.

Li X., Gibson G., Kim J.S., Kroin J., Xu S., Wijnen A.J. and Im H.J. (2011). MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. Gene 480, 34-41.

Li H., Gu Z., Wu L., Xia L., Zhou K., E L., Wang D., Kou J. and Liu H. (2014). Danggui-shaoyao-san, a traditional Chinese medicine prescription, alleviates the orthodontic pain and inhibits neuronal and microglia activation. Chin. Med. J. (Engl). 127, 3630-3637.

Li L., Li L., Chen C., Yang J., Li J., Hu N., Li Y., Zhang D., Guo T., Liu X. and Yang W. (2015). Scutellarin's cardiovascular endothelium protective mechanism: Important role of PKG-Iα. PLoS One 10, e0139570.

Li Z., Mo N., Li L., Cao Y., Wang W., Liang Y., Deng H., Xing R., Yang L., Ni C., Chui D. and Guo X. (2016). Surgery-induced hippocampal angiotensin II elevation causes blood-brain barrier disruption via MMP/TIMP in aged rats. Front. Cell. Neurosci. 10, 105-105.

Li T.F., Wu H.Y., Wang Y.R., Li X.Y. and Wang Y.X. (2017). Molecular signaling underlying bulleyaconitine A (BAA)-induced microglial expression of prodynorphin. Sci. Rep. 7, 45056-45056.

Liang H., Chen A., Lai X., Liu J., Wu J., Kang Y., Wang X. and Shao L. (2018). Neuroinflammation is induced by tongue-instilled ZnO nanoparticles via the Ca2+-dependent NF-κB and MAPK pathways. Part. Fibre. Toxicol. 15, 39-39.

Lin S.J. and Guarente L. (2003). Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. Curr. Opin. Cell. Biol. 15, 241-246.

Lin L.C., Chen Y.F., Tsai T.R. and Tsai T.H. (2007a). Analysis of brain distribution and biliary excretion of a nutrient supplement, gastrodin, in rat. Anal. Chim. Acta 590, 173-179.

Lin L.L., Liu A.J., Liu J.G., Yu X.H., Qin L.P. and Su D.F. (2007b). Protective effects of scutellarin and breviscapine on brain and heart ischemia in rats. J. Cardiovasc. Pharmacol. 50, 327-332.

Lin L.C., Chen Y.F., Lee W.C., Wu Y.T. and Tsai T.H. (2008). Pharmacokinetics of gastrodin and its metabolite p-hydroxybenzyl alcohol in rat blood, brain and bile by microdialysis coupled to LC-MS/MS. J. Pharm. Biomed. Anal. 48, 909-917.

Lin C.J., Chen T.H., Yang L.Y. and Shih C.M. (2014). Resveratrol protects astrocytes against traumatic brain injury through inhibiting apoptotic and autophagic cell death. Cell. Death Dis. 5, e1147.

Liu H., Yang X., Tang R., Liu J. and Xu H. (2005). Effect of scutellarin on nitric oxide production in early stages of neuron damage induced by hydrogen peroxide. Pharmacol. Res. 51, 205-210.

Liu H., Chen T., Li N., Wang S. and Bu P. (2015). Role of SIRT3 in Angiotensin II-induced human umbilical vein endothelial cells dysfunction. BMC. Cardiovasc. Disord. 15, 81-81.

Liu B., Li F., Shi J., Yang D., Deng Y. and Gong Q. (2016). Gastrodin ameliorates subacute phase cerebral ischemia reperfusion injury by inhibiting inflammation and apoptosis in rats. Mol. Med. Rep. 14, 4144-4152.

Liu X.S., Chopp M., Pan W.L., Wang X.L., Fan B.Y., Zhang Y., Kassis H., Zhang R.L., Zhang X.M. and Zhang Z.G. (2017). MicroRNA-146a promotes oligodendrogenesis in stroke. Mol. Neurobiol. 54, 227-237.

Liu S.J., Liu X.Y., Li J.H., Guo J., Li F., Gui Y., Li X.H., Yang L., Wu C.Y., Yuan Y. and Li J.J. (2018). Gastrodin attenuates microglia activation through renin-angiotensin system and Sirtuin3 pathway. Neurochem. Int. 120, 49-63.

Locatelli F.M., Kawano T., Iwata H., Aoyama B., Eguchi S., Nishigaki A., Yamanaka D., Tateiwa H., Shigematsu-Locatelli M. and Yokoyama

M. (2018). Resveratrol-loaded nanoemulsion prevents cognitive decline after abdominal surgery in aged rats. J. Pharmacol. Sci. 137, 395-402.

Lofrumento D.D., Nicolardi G., Cianciulli A., Nuccio F.D., Pesa V.L, Carofiglio V., Dragone T., Calvello R. and Panaro M.A. (2014). Neuroprotective effects of resveratrol in an MPTP mouse model of Parkinson's-like disease: Possible role of SOCS-1 in reducing pro-inflammatory responses. Innate Immun. 20, 249-260.

Long L., Wang J., Lu X., Xu Y., Zheng S., Luo C. and Li Y. (2015). Protective effects of scutellarin on type II diabetes mellitus-induced testicular damages related to reactive oxygen species/Bcl-2/Bax and reactive oxygen species/microcirculation/staving pathway in diabetic rat. J. Diabetes Res. 2015, 252530.

Lu D.Y., Liou H.C., Tang C.H. and Fu W.M. (2006). Hypoxia-induced iNOS expression in microglia is regulated by the PI3-kinase/Akt/mTOR signaling pathway and activation of hypoxia inducible factor-1alpha. Biochem. Pharmacol. 72, 992-1000.

Lu X., Ma L., Ruan L., Kong Y., Mou H., Zhang Z., Wang Z., Wang J.M. and Le Y. (2010). Resveratrol differentially modulates inflammatory responses of microglia and astrocytes. J. Neuroinflammation 7, 46-46.

Lu Y., Cao D.L., Jiang B.C., Yang T. and Gao Y.J. (2015). MicroRNA-146a-5p attenuates neuropathic pain via suppressing TRAF6 signaling in the spinal cord. Brain Behav. Immun. 49, 119-129.

Lull M.E. and Block M.L. (2010). Microglial activation and chronic neurodegeneration. Neurotherapeutics 7, 354-365.

Luo L.J., Liu F., Lin Z.K., Xie Y.F., Xu J.L., Tong Q.C.and Shu R. (2012). Genistein regulates the IL-1 beta induced activation of MAPKs in human periodontal ligament cells through G protein-coupled receptor 30. Arch. Biochem. Biophys. 522, 9-16.

Luo Q., Yan X., Bobrovskaya L., Ji M., Yuan H., Lou H. and Fan P. (2017). Anti-neuroinflammatory effects of grossamide from hemp seed via suppression of TLR-4-mediated NF-κB signaling pathways in lipopolysaccharide-stimulated BV2 microglia cells. Mol. Cell. Biochem. 428, 129-137.

Marchetti B. and Pluchino S. (2013). Wnt your brain be inflamed? Yes, it Wnt! Trends. Mol. Med. 19, 144-156.

Markus M.A. and Morris B.J. (2008). Resveratrol in prevention and treatment of common clinical conditions of aging. Clin. Interv. Aging 3, 331-339.

Martin M., Rehani K., Jope R.S. and Michalek S.M. (2005). Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase. Nat. Immunol. 6, 777-784.

Mei J., Bachoo R. and Zhang C.L. (2011). MicroRNA-146a inhibits glioma development by targeting Notch1. Mol. Cell. Biol. 31, 3584-3592.

Mei X., Zhang T., Ouyang H., Lu B., Wang Z. and Ji L. (2019). Scutellarin alleviates blood-retina barrier oxidative stress injury initiated by activated microglia cells during the development of diabetic retinopathy. Biochem. Pharmacol. 159, 82-95.

Meng D., Frank A.R., and Jewell J.L. (2018). mTOR signaling in stem and progenitor cells. Development 145, dev152595.

Millan M.A., Kiss A. and Aguilera G. (1991). Developmental changes in brain angiotensin II receptors in the rat. Peptides 12, 723-737.

Moussa C., Hebron M., Huang X., Ahn J., Rissman R.A., Aisen P.S. and Turner R.S. (2017). Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's disease. J. Neuroinflammation 14, 1-1.

Nah S.Y., Kim D.H. and Rhim H. (2007). Ginsenosides: are any of them candidates for drugs acting on the central nervous system? CNS Drug Rev. 13, 381-404.

Nam N.H. (2006). Naturally occurring NF-kappaB inhibitors. Mini. Rev. Med. Chem. 6, 945-951.

Nishimura Y., Ito T. and Saavedra J.M. (2000). Angiotensin II AT(1) blockade normalizes cerebrovascular autoregulation and reduces cerebral ischemia in spontaneously hypertensive rats. Stroke 31, 2478-2486.

Niu Q., Niu P. and He S. (2004). Effect of gastrodia elata on learning and memory impairment induced by aluminum in rats. Wei. Sheng. Yan. Jiu. 33, 45-48.

Ock J., Kim S. and Suk K. (2009). Anti-inflammatory effects of a fluorovinyloxyacetamide compound KT-15087 in microglia cells. Pharmacol. Res. 59, 414-422.

Oeckinghaus A., Hayden M.S. and Ghosh S. (2011). Crosstalk in NF-kappaB signaling pathways. Nat. Immunol. 12, 695-708.

Onyango P., Celic I., McCaffery J.M., Boeke J.D. and Feinberg A.P. (2002). SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. Proc. Natl. Acad. Sci. USA 99, 13653-13658.

Orsu P., Murthy B.V. and Akula A. (2013). Cerebroprotective potential of resveratrol through anti-oxidant and anti-inflammatory mechanisms in rats. Neural. Transm. 120, 1217-1223.

Pan Y., Wang J., Xue Y., Zhao J., Li D., Zhang S., Li K., Hou Y. and Fan H. (2018). GSKJ4 protects mice against early sepsis via reducing proinflammatory factors and up-regulating MiR-146a. Front. Immunol. 9, 2272-2272.

Park S., Kim D.S. and Kang S. (2011). *Gastrodia elata* Blume water extracts improve insulin resistance by decreasing body fat in diet-induced obese rats: vanillin and 4-hydroxybenzaldehyde are the bioactive candidates. Eur. J. Nutr. 50, 107-118.

Parkhurst C.N. and Gan W.B. (2010). Microglia dynamics and function in the CNS. Curr. Opin. Neurobiol. 20, 595-600.

Patnala R., Arumugam T.V., Gupta N. and Dheen S.T. (2017). HDAC inhibitor sodium butyrate-mediated epigenetic regulation enhances neuroprotective function of microglia during ischemic stroke. Mol. Neurobiol. 54, 6391-6411.

Patterson R.L., Rossum D.B., Nikolaidis N., Gill DL. and Snyder S.H. (2005). Phospholipase C-gamma: diverse roles in receptor-mediated calcium signaling. Trends Biochem. Sci. 30, 688-697.

Pearson G., Robinson F., Gibson B.T., Xu B.E., Karandikar M., Berman K. and Cobb M.H. (2001). Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr. Rev. 22, 153-183.

Revankar C.M., Cimino D.F., Sklar L.A., Arterburn J.B. and Prossnitz E.R. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307, 1625-1630.

Robb E.L, Winkelmolen L, Visanji N, Brotchie J and Stuart J.A. (2008). Dietary resveratrol administration increases MnSOD expression and activity in mouse brain. Biochem. Biophys. Res. Commun. 372, 254-259.

Rose G., Dato S., Altomare K., Bellizzi D., Garasto S., Greco V., Passarino G., Feraco E., Mari V., Barbi C., BonaFe M., Franceschi C., Tan Q., Boiko S., Yashin A.I. and Benedictis G.D. (2003). Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. Exp. Gerontol. 38, 1065-1070.

Russo C.D., Lisi L., Tringali G. and Navarra P. (2009). Involvement of mTOR kinase in cytokine-dependent microglial activation and cell

proliferation. Biochem. Pharmacol. 78, 1242-1251.

Saccani S., Pantano S. and Natoli G. (2002). P38-dependent marking of inflammatory genes for increased NF-kappa B recruitment. Nat. Immunol. 3, 369-375.

Saxton R.A. and Sabatini D.M. (2017). mTOR signaling in growth, metabolism, and disease. Cell 169, 361-371.

Schwarzer R., Dorken B. and Jundt F. (2012). Notch is an essential upstream regulator of NF-κB and is relevant for survival of Hodgkin and Reed-Sternberg cells. Leukemia 26, 806-813.

Shahal B., Piel E., Mecz L., Kremer I. and Klein E. (1996). Lack of advantage for imipramine combined with lithium versus imipramine alone in the treatment of major depression-a double-blind controlled study. Biol. Psychiatry 40, 1181-1183.

Shakibaei M., Csaki C., Nebrich S. and Mobasheri A. (2008). Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochem. Pharmacol. 76, 1426-1439.

Shakibaei M., Harikumar K.B. and Aggarwal B.B. (2009). Resveratrol addiction: to die or not to die. Mol. Nutr. Food. Res. 53, 115-128.

Sharma N., Verma R., Kumawat K.L., Basu A., and Singh S.K. (2015). miR-146a suppresses cellular immune response during Japanese encephalitis virus JaOArS982 strain infection in human microglial cells. J. Neuroinflammation 12, 30-30.

Sheu J.N., Liao W.C., Wu U.I., Shyu L.Y., Mai F.D., Chen L.Y., Chen M.J., Youn S.C. and Chang H.M. (2013). Resveratrol suppresses calcium-mediated microglial activation and rescues hippocampal neurons of adult rats following acute bacterial meningitis. Comp. Immunol. Microbiol. Infect. Dis. 36, 137-148.

Shi D.D., Huang Y.H., Lai C.S.W., Dong C.M., Ho L.C., Li X.Y., Wu E.X., Li Q., Wang X.M., Chen Y.J., Chung S.K. and Zhang Z.J. (2019). Ginsenoside Rg1 prevents chemotherapy-induced cognitive impairment: associations with microglia-mediated cytokines, neuroinflammation, and neuroplasticity. Mol. Neurobiol. 56, 5626-5642.

Shin J.W., Kweon K.J., Kim D.K., Kim P., Jeon T.D., Maeng S. and Sohn N.W. (2018). Scutellarin ameliorates learning and memory deficit via suppressing β-Amyloid formation and microglial activation in rats with chronic cerebral hypoperfusion. Am. J. Chin. Med. 46, 1203-1223.

Solt L.A., Madge L.A., Orange J.S. and May M.J. (2007). Interleukin-1-induced NF-kappaB activation is NEMO-dependent but does not require IKKbeta. J. Biol. Chem. 282, 8724-8733.

Song J.Z., Li L.J., Ji L., Shun L. and Rui Y. (2017). The pharmacokinetics of Tiangou antihypertensive capsule in rat *in vivo*. Biomed. Rep. 6, 113-119.

Song X.Y., Hu J.F., Chu S.F., Zhang Z., Xu S., Yuan Y.H., Han N., Liu Y., Niu F., He X. and Chen N.H. (2013). Ginsenoside Rg1 attenuates okadaic acid induced spatial memory impairment by the GSK3β/tau signaling pathway and the Aβ formation prevention in rats. Eur. J. Pharmacol. 710, 29-38.

Stambolic V., Ruel L. and Woodgett J.R. (1997). Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. Curr. Biol. 7, 196-196.

Stoll M. and Unger T. (2001). Angiotensin and its AT2 receptor: new insights into an old system. Regul. Pept. 99, 175-182.

Sun W., Miao B., Wang X.C., Duan J.H., Ye X., Han W.J., Wang W.T., Luo C. and Hu S.J. (2012a). Gastrodin inhibits allodynia and hyperalgesia in painful diabetic neuropathy rats by decreasing excitability of nociceptive primary sensory neurons. PLoS One 7, e39647.

Sun Y.Y., Li L., Liu X.H., Gu N., Dong H.L. and Xiong L. (2012b). The spinal notch signaling pathway plays a pivotal role in the development of neuropathic pain. Mol. Brain. 5, 23-23.

Tamaki M., Konno Y., Kobayashi Y., Takeda M., Itoga M., Moritoki Y., Oyamada H., Kayaba H., Chihara J. and Ueki S. (2014). Expression and functional roles of G-protein-coupled estrogen receptor (GPER) in human eosinophils. Immunol. Lett. 160, 72-78.

Tang H., Tang Y., Li N., Shi Q., Guo J., Shang E. and Duan J.A. (2014). Neuroprotective effects of scutellarin and scutellarein on repeatedly cerebral ischemia-reperfusion in rats. Pharmacol. Biochem. Behav. 118, 51-59.

Tao R., Coleman M.C., Pennington J.D., Ozden O., Park S.H., Jiang H., Kim H.S., Flynn C.R., Hill S., McDonald W.H., Olivier A.K., Spitz D.R. and Gius D. (2010). Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. Mol. Cell 40, 893-904.

Tawab M.A., Bahr U., Karas M., Wurglics M. and Schubert-Zsilavecz M. (2003). Degradation of ginsenosides in humans after oral administration. Drug Metab. Dispos. 31, 1065-1071.

Thomson A.W., Turnquist H.R. and Raimondi G. (2009). Immunoregulatory functions of mTOR inhibition. Nat. Rev. Immunol. 9, 324-337.

Tsoyi K., Kim W.S., Kim Y.M., Kim H.J., Seo H.G., Lee J.H., Yun-Choi H.S. and Chang K.C. (2009). Upregulation of PTEN by CKD712, a synthetic tetrahydroisoquinoline alkaloid, selectively inhibits lipopolysaccharide-induced VCAM-1 but not ICAM-1 expression in human endothelial cells. Atherosclerosis 207, 412-419.

Tsutsumi K., Viswanathan M., Strömberg C. and Saavedra J.M. (1991). Type-1 and type-2 angiotensin II receptors in fetal rat brain. Eur. J. Pharmacol. 198, 89-92.

Turner R.S., Thomas R.G., Craft S., Dyck C.H., Mintzer J., Reynolds B.A., Brewer J.B., Rissman R.A., Raman R. and Aisen P.S. (2015). A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease. Neurology 85, 1383-1391.

Vaillancourt F., Morquette B., Shi Q., Fahmi H., Lavigne P., Battista J.A.D., Fernandes J.C. and Benderdour M. (2007). Differential regulation of cyclooxygenase-2 and inducible nitric oxide synthase by 4-hydroxynonenal in human osteoarthritic chondrocytes through ATF-2/ CREB-1 transactivation and concomitant inhibition of NF-kappaB signaling cascade. J. Cell. Biochem. 100, 1217-1231.

Vazquez F., Ramaswamy S., Nakamura N. and Sellers W.R. (2000). Phosphorylation of the PTEN tail regulates protein stability and function. Mol. Cell. Biol. 20, 5010-5018.

Venneti S., Wiley C.A. and Kofler J. (2009). Imaging microglial activation during neuroinflammation and Alzheimer's disease. J. NeuroImmune. Pharmacol. 4, 227-243.

Vitrac X., Desmoulière A., Brouillaud B., Krisa S., Deffieux G., Barthe N., Rosenbaum J. and Mérillon J.M. (2003). Distribution of [14C]-trans-resveratrol, a cancer chemopreventive polyphenol, in mouse tissues after oral administration. Life. Sci. 72, 2219-2233.

Wang J.Y., Gualco E., Peruzzi F., Sawaya B.E., Passiatore G., Marcinkiewicz C., Staniszewska I., Ferrante P., Amini S., Khalili K. and Reiss K. (2007). Interaction between serine phosphorylated IRS-1 and beta1-integrin affects the stability of neuronal processes. J. Neurosci. Res. 85, 2360-2373.

Wang H., Brown J., Gu Z., Garcia C.A., Liang R., Alard P., Beurel E., Jope R.S., Greenway T. and Martin M. (2011a). Convergence of the

mammalian target of rapamycin complex 1- and glycogen synthase kinase 3-β-signaling pathways regulates the innate inflammatory response. J. Immunol. 186, 5217-5226.

Wang S., Wang H., Guo H., Kang L., Gao X. and Hu L. (2011b). Neuroprotection of Scutellarin is mediated by inhibition of microglial inflammatory activation. Neuroscience 185, 150-160.

Wang Y., Ao H., Qian Z. and Zheng Y. (2011c). Intestinal transport of scutellarein and scutellarin and first-pass metabolism by UDP-glucuronosyltransferase-mediated glucuronidation of scutellarein and hydrolysis of scutellarin. Xenobiotica 41, 538-548.

Wang X., Ha T., Liu L., Zou J., Zhang X., Kalbfleisch J., Gao X., Williams D. and Li C. (2013). Increased expression of microRNA-146a decreases myocardial ischaemia/reperfusion injury. Cardiovasc. Res. 97, 432-442.

Wang W., Ma X., Han J., Zhou M., Ren H., Pan Q., Zheng C. and Zheng Q. (2016). Neuroprotective effect of scutellarin on ischemic cerebral injury by down-regulating the expression of angiotensin converting enzyme and AT1 receptor. PLoS One 11, e0147780.

Wei Z., Chigurupati S., Arumugam T.V., Jo D.G., Li H. and Chan S.L. (2011). Notch activation enhances the microglia-mediated inflammatory response associated with focal cerebral ischemia. Stroke 42, 2589-2594.

Weichhart T., Costantino G., Poglitsch M., Rosner M., Zeyda M., Stuhlmeier K.M., Kolbe T., Stulnig T.M. and Hörl W.H. (2008). The TSC-mTOR signaling pathway regulates the innate inflammatory response. Immunity 29, 565-577.

Whitmarsh A.J. (2007). Regulation of gene transcription by mitogenactivated protein kinase signaling pathways. Biochim. Biophys. Acta 1773, 1285-1298.

Wu C.F., Bi X.L., Yang J.Y., Zhan J.Y., Dong Y.X., Wang J.H., Wang J.M., Zhang R. and Li X. (2007). Differential effects of ginsenosides on NO and TNF-alpha production by LPS activated N9 microglia. Int. Immunopharmacol. 7, 313-320.

Wu C.Y., Zha H., Xia Q.Q., Yuan Y., Liang X.Y., Li J.H., Guo Z.Y. and Li J.J. (2013). Expression of angiotensin II and its receptors in activated microglia in experimentally induced cerebral ischemia in the adult rats. Mol. Cell. Biochem. 382, 47-58.

Wu L., Li Y., Yu M., Yang F., Tu M. and Xu H. (2018). Notch signaling regulates microglial activation and inflammatory reactions in a rat model of temporal lobe epilepsy. Neurochem. Res. 43, 1269-1282.

Xiao M.M., Zhang Y.Q., Wang W.T., Han W.J., Lin Z., Xie R.G., Cao Z., Lu N., Hu S.J., Wu S.X., Dong H. and Luo C. (2016). Gastrodin protects against chronic inflammatory pain by inhibiting spinal synaptic potentiation. Sci. Rep. 6, 37251-37251.

Xu T.M., Xin Y., Cui M.H., Jiang X. and Gu L.P. (2007). Inhibitory effect of ginsenoside Rg3 combined with cyclophosphamide on growth and angiogenesis of ovarian cancer. Chin. Med. J. (Engl). 120, 584-588.

Yan J., Liu Q., Dou Y., Hsieh Y., Liu Y., Tao R., Zhu D. and Lou Y. (2013). Activating glucocorticoid receptor-ERK signaling pathway contributes to ginsenoside Rg1 protection against β-amyloid peptide-induced human endothelial cells apoptosis. J. Ethnopharmacol. 147, 456-466.

Yang X.D., Zhu J., Yang R., Liu J.P., Li L. and Zhang H.B. (2007). Phenolic constituents from the rhizomes of *Gastrodia elata*. Nat. Prod. Res. 21, 180-186.

Yang T., Fan G.H. and Diao B. (2013). Influence of Gastrodin sustained release tablets on CD4+ CD25+ regulatory T cells and interleukin-17 in patients with high-risk transient ischemic attack in posterior circulation. Herald. Med. 32, 1583-1586.

Yao L., Cao Q., Wu C., Kaur C., Hao A. and Ling E.A. (2013a). Notch signaling in the central nervous system with special reference to its expression in microglia. CNS Neurol. Disord. Drug. Targets 12, 807-814.

Yao L., Kan E.M., Kaur C., Dheen S.T., Hao A., Lu J. and Ling E.A. (2013b). Notch-1 signaling regulates microglia activation via NF-κB pathway after hypoxic exposure *in vivo* and *in vitro*. PLoS One 8, e78439.

Yao Y.Y., Bian L.G., Yang P., Sui Y., Li R., Chen Y.L., Sun L., Ai Q.L., Zhong L.M. and Lu D. (2019). Gastrodin attenuates proliferation and inflammatory responses in activated microglia through Wnt/β-catenin signaling pathway. Brain Res. 1717, 190-203.

Yong W., Xing T.R., Wang S., Chen L., Hu P., Li C.C., Wang H.L., Wang M., Chen J.T. and Ruan D.Y. (2009). Protective effects of gastrodin on lead-induced synaptic plasticity deficits in rat hippocampus. Planta Med. 75, 1112-1117.

Yu S.J., Kim J.R., Lee C.K., Han J.E., Lee J.H., Kim H.S., Hong J.H. and Kang S.G. (2005). *Gastrodia elata* blume and an active component, p-hydroxybenzyl alcohol reduce focal ischemic brain injury through antioxidant related gene expressions. Biol. Pharm. Bull. 28, 1016-1020.

Yuan W., Guo J., Li X., Zou Z., Chen G., Sun J., Wang T. and Lu D. (2009). Hydrogen peroxide induces the activation of the phospholipase C-1 survival pathway in PC12 cells: protective role in apoptosis. Acta. Biochim. Biophys. Sin. (Shanghai) 41, 625-630.

Yuan Y., Zha H., Rangarajan P., Ling E.A. and Wu C. (2014). Anti-inflammatory effects of edaravone and scutellarin in activated microglia in experimentally induced ischemia injury in rats and in BV-2 microglia. BMC Neurosci. 15, 125-146.

Yuan Y., Rangarajan P., Kan E., Wu Y., Wu C. and Ling E.A. (2015). Scutellarin regulates the Notch pathway and affects the migration and morphological transformation of activated microglia in experimentally induced cerebral ischemia in rats and in activated BV-2 microglia. J. Neuroinflammation 12, 11.

Yuan Y., Fang M., Wu C.Y. and Ling E.A. (2016). Scutellarin as a potential therapeutic agent for microglia-mediated neuroinflammation in cerebral ischemia. Neuromolecular Med. 18, 264-273.

Yuan Y., Wu C. and Ling E.A. (2019). Heterogeneity of Microglia Phenotypes: Developmental, Functional and Some Therapeutic Considerations. Curr. Pharm. Des. 25, 2375-2393.

Yue P.Y., Wong D.Y., Wu P.K., Leung P.Y., Mak N.K., Yeung H.W., Liu L., Cai Z., Jiang Z.H., Fan T.P. and Wong R.N. (2006). The angiosuppressive effects of 20(R)-ginsenoside Rg3. Biochem. Pharmacol. 72, 437-445.

Zawada W.M., Mrak R.E., Biedermann J., Palmer Q.D., Gentleman S.M., Aboud O. and Griffin W.S. (2015). Loss of angiotensin II receptor expression in dopamine neurons in Parkinson's disease correlates with pathological progression and is accompanied by increases in Nox4- and 8-OH guanosine-related nucleic acid oxidation and caspase-3 activation. Acta Neuropathol. Commun. 3, 9-25.

Zawadzka M. and Kaminska B. (2005). A novel mechanism of FK506-mediated neuroprotection: downregulation of cytokine expression in glial cells. Glia 49, 36-51.

Zhang H.F., Hu X.M., Wang L.X., Xu S.Q. and Zeng F.D. (2009). Protective effects of scutellarin against cerebral ischemia in rats:

evidence for inhibition of the apoptosis-inducing factor pathway. Planta Medica 75, 121-126.

Zhang Q., Wang C., Liu Z., Liu X., Han C., Cao X. and Li N. (2012). Notch signal suppresses Toll-like receptor-triggered inflammatory responses in macrophages by inhibiting extracellular signal-regulated kinase 1/2-mediated nuclear factor κB activation. J. Biol. Chem. 287, 6208-6217.

Zhang X.S., Li W., Wu Q., Wu L.Y., Ye Z.N., Liu J.P., Zhuang Z., Zhou M.L., Zhang X. and Hang C.H. (2016). Resveratrol attenuates acute inflammatory injury in experimental subarachnoid hemorrhage in rats via inhibition of TLR4 pathway. Int. J. Mol. Sci. 17, e1131.

Zhao T.Z., Ding Q., Hu J., He S.M., Shi F. and Ma L.T. (2016). GPER expressed on microglia mediates the anti-inflammatory effect of estradiol in ischemic stroke. Brain Behav. 6, e00449.

Zhao X., Gao J., Song C., Fang Q., Wang N., Zhao T., Liu D. and Zhou Y. (2012). Fungal sensitivity to and enzymatic deglycosylation of ginsenosides. Phytochemistry 78, 65-71.

Zhong L.M., Zong Y., Sun L., Guo J.Z., Zhang W., He Y., Song R., Wang W.M., Xiao C.J. and Lu D. (2012). Resveratrol inhibits inflammatory responses via the mammalian target of rapamycin signaling pathway in cultured LPS-stimulated microglial cells. PLoS One 7, e32195.

Zhou H.Y., Shin E.M., Guo L.Y., Youn U.J., Bae K., Kang S.S., Zou L.B. and Kim Y.S. (2008). Anti-inflammatory activity of 4-methoxyhonokiol is a function of the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via NF-kappaB, JNK and p38 MAPK inactivation. Eur. J. Pharmacol. 586, 340-349.

Zhou Y., Li H.Q., Lu L., Fu D.L., Liu A.J., Li J.H. and Zheng G.Q. (2014). Ginsenoside Rg1 provides neuroprotection against blood brain barrier disruption and neurological injury in a rat model of cerebral ischemia/reperfusion through downregulation of aquaporin 4 expression. Phytomedicine 21, 998-1003.

Zhou T.T., Zu G., Wang X., Zhang X.G., Li S., Liang Z.H. and Zhao J. (2015). Immunomodulatory and neuroprotective effects of ginsenoside Rg1 in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced mouse model of Parkinson's disease. Int. Immunopharmacol. 29, 334-343.

Zhu S., Zou K., Cai S., Meselhy M.R. and Komatsu K. (2004). Simultaneous determination of triterpene saponins in ginseng drugs by high-performance liquid chromatography. Chem. Pharm. Bull. (Tokyo). 52, 995-998.

Zhu P., Liao L.Y., Zhao T.T., Mo X.M. and Chen G.G. (2017). GPER/ERK&AKT/NF-κB pathway is involved in cadmium-induced proliferation, invasion and migration in GPER-positive thyroid cancer cells. Mol. Cell. Endocrinol. 422, 68-80.

Zong Y., Ai Q.L., Zhong L.M., Dai J.N., Yang P., He Y., Sun J., Ling E.A. and Lu D. (2012). Ginsenoside Rg1 attenuates lipopolysaccharide-induced inflammatory responses via the phospholipase C-1 signaling pathway in murine BV-2 microglial Cells. Curr. Med. Chem. 19, 770-779.

Accepted July 14, 2020