Regulation of Neuroinflammation by Herbal Medicine and Its Implications for Neurodegenerative Diseases

A Focus on Traditional Medicines and Flavonoids

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Key Words

Inflammation · Neuroglia · Neurodegenerative disease · Flavonoid · Neuroprotection · Microglia · Astrocyte · Central nervous system

Abstract

Herbal medicine has long been used to treat neural symptoms. Although the precise mechanisms of action of herbal drugs have yet to be determined, some of them have been shown to exert anti-inflammatory and/or antioxidant effects in a variety of peripheral systems. Now, as increasing evidence indicates that neuroglia-derived chronic inflammatory responses play a pathological role in the central nervous system, anti-inflammatory herbal medicine and its constituents are being proved to be a potent neuroprotector against various brain pathologies. Structural diversity of medicinal herbs makes them valuable source of novel lead compounds against therapeutic targets that are newly discovered by genomics, proteomics, and high-throughput screening.

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Introduction

In neuroinflammation, microglia and astrocytes play a critical role. Microglial cells are ubiquitously distributed in the central nervous system (CNS) and comprise up to 20% of the total glial cell population in brain [1, 2]. Although the ontogeny of microglial cells has long been debated, recent works using monoclonal antibodies specific for microglial cells indicated that these cells are closely related to monocytes and macrophages [3]. As the primary immune effector cells in the CNS, microglial cells migrate to the site of tissue injury or inflammation, where they respond to invading pathogens or other inflammatory signals [4, 5]. Like monocytes/macrophages, they also secrete inflammatory cytokines and toxic mediators which may amplify the neuroinflammatory responses [6, 7]. Astrocytes form an intimately connected network with neurons in the CNS, and they provide mechanical and metabolic support for neurons [8]. The critical role of these cells in ion buffering and clearance of neurotransmitters is also well established [9, 10]. Upon inflammatory stimulation, astrocytes proliferate and produce diverse intercellular mediators such as nitric oxide (NO) and tumor necrosis factor (TNF)- α [11–13]. There is growing evidence that inflammatory mediators produced by activated astrocytes may be involved in the pathogenesis of various neurodegenerative diseases [10, 14]. Thus,

Fig. 1. Relationship between inflammation and tissue injury. Tissue damage can be triggered by a number of genetic or environmental factors. While reversible tissue damages could be repaired for a functional recovery, irreversible damages associated with inappropriately controlled inflammation (chronic inflammation) could lead to diseases.

the activation of astrocytes and ensuing production of toxic inflammatory mediators may need to be tightly regulated. Activation of inflammatory cells in CNS (microglia or astrocytes) may be intended to protect neurons at first. More frequently, however, activation of these neuroglial cells and inflammatory products derived from them have been implicated in neuronal destruction commonly observed in various neurodegenerative diseases [7]. Thus, our understanding of pathogenesis of neurodegenerative diseases may be enhanced by elucidation of the molecular mechanism underlying the regulation of neuroglial activation. Among many endogenous or exogenous factors that regulate neuroglial activation and resulting neuroinflammation [15], herbal medicine has recently drawn much attention due to its potent inhibitory effects on inflammatory responses and neuroprotective activity [16, 17]. A central role of microglia and astrocytes in neuroinflammation (and potentially neurodegeneration) and a regulatory effect of herbal medicine on the inflammatory activation of the neuroglia will be discussed in this review.

Inflammation and Tissue Injury

Injury, trauma or infection induce a series of complex and interconnected reaction sequences, initiated at the site of tissue damage [18, 19]. This sequence of reaction

serves to contain and destroy the infection or damaging agents, and to prevent continued tissue damage and initiate repair processes to restore normal function. This rapid response is known as acute inflammation [20]. The toxic reactions, which are employed to destroy infectious organisms or protect host, also paradoxically have the capacity to injure host tissues. If these toxic responses are not tightly regulated, tissue injury may predominate over tissue protection and repair, thereby leading to inflammatory diseases (fig. 1). The characteristics of the inflammatory response include localized changes within the damaged tissue such as the followings: (1) the release of pre-formed inflammatory mediators from intracellular stores; (2) the initiation of reaction cascade through the activation of soluble plasma components; (3) the new synthesis of inflammatory mediators such as eicosanoids and cytokines, and (4) resolution of the inflammatory response. The acute inflammatory response is beneficial to the organism in that it helps to deal with potentially dangerous microorganisms. However, inflammation does cause some degree of damage to surrounding tissues. Reactive oxygen species (ROS), reactive nitrogen species (RNS), prostanoids, leukotrienes, and hydrolytic enzymes produced by neutrophils, macrophages, and monocytes may all play a role in mediating inflammation. Persistence of infection or defective resolution of inflammatory reaction results in chronic inflammation where severe tissue damage may occur. Although inflammation is normally a self-

Fig. 2. Neuroprotective versus neurotoxic activities of neuroglia. Neuroglial activation is induced by a variety of intrinsic or extrinsic factors. Physiological neuroglial activation is beneficial and designed to ameliorate damage and stress. In contrast, pathological activation of neuroglia leads to neurotoxicity. Apoptotic elimination of overactivated neuroglia represents a self-regulatory mechanism. When the auto-regulation is at work, physiological neuroprotection may allow a neuroregeneration. On the contrary, a defective regulation may cause neurodegeneration.

limiting event and its benefit outweighs the minor tissue damage it causes, abnormal activation of the immune or inflammatory system has the potential to provoke a devastating response [21]. In gout, for example, elevated concentration of uric acid in the blood leads to precipitation of sodium urate crystal within joints which triggers inflammation by a variety of mechanisms. Another striking consequence of abnormal inflammatory response is autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, autoimmune vasculitis, dermatomyositis, chronic autoimmune gastritis, and myasthenia gravis. Tissue-damaging chronic inflammatory response may also occur in CNS, where main inflammatory cells are microglia and astrocytes instead of monocytes/macrophages or neutrophils in periphery [22–24].

Neuroglia (Microglia, Astrocytes), Neuroinflammation, and Neurodegeneration

Microglia and astrocytes are essential for ensuring proper functioning of neurons. They are quick to intervene when neurons become injured or stressed. As they are sentinels of neuron well-being, pathological impairment of microglia or astrocytes could have devastating consequences for brain function. Nevertheless, there is still a debate over neuroprotective and neurotoxic functions of these neuroglial cells [22, 25] (fig. 2). It is assumed that neuroglial activation is largely determined by neuronal signals. Acute injury causes neurons to generate signals that inform neuroglia about the neuronal status. Depending on how severe a degree of neuronal injury, neu-

roglia will either nurse the injured neurons into regeneration or kill them if they are not viable. These types of neuroglial responses are considered to represent normal physiological and neuroprotective responses. In contrast, some processes that are chronic in nature persistently activate neuroglia eventually causing a failure in their physiological ability to maintain homeostasis. This could have detrimental consequences and may lead to bystander damage due to neuroglial dysfunction. In this scenario, neuroglia exert neurotoxic effects through the secretion of a variety of toxic inflammatory mediators. Thus, although activation of neuroglial cells may be intended to protect neurons, inflammatory products derived from activated neuroglia may also be implicated in neuronal injury, potentially leading to neurodegenerative diseases [7]. These deleterious effects of neuroglial activation may be exacerbated by the failure of auto-regulatory mechanisms of neuroglia. Recently, activated macrophages, whose functions are closely related to microglia, have been shown to undergo apoptosis [26–28]. It has been suggested that the apoptosis of activated macrophages is one mechanism whereby an organism may regulate immune and inflammatory responses involving macrophages [28]. It has been recently demonstrated that a similar regulatory mechanism exists for microglial cells [29, 30] and astrocytes [31] as well. Microglial cells and astrocytes underwent apoptosis upon inflammatory activation in a manner similar to activation-induced cell death (AICD) of lymphocytes [30, 31]. AICD is an active process. T and B lymphocytes undergo AICD as an auto-regulatory mechanism for the body to remove unwanted activated cells after making appropriate use of them [32,

33]. Compared to lymphocytes, neuroglial cells in CNS are not well studied in this respect. Now, as results in this and other laboratories indicated that neuroglial cells might be under the control of a similar regulatory mechanism [29–31, 34–37], further investigation is warranted to better understand the molecular mechanism(s) of neuroglial AICD and its physiological significance. Nevertheless, it has been shown that, in contrast to AICD of T lymphocytes where Fas-FasL interaction plays a central role, neither Fas-FasL interaction nor TNF- α is important in AICD of microglial cells [30]. Instead, NO produced by activated neuroglial cells themselves was the major cytotoxic mediator [30, 31]. However, the presence of NO-independent cytotoxic mechanism has been also suggested [38, 39].

Elimination of activated neuroglial cells by apoptosis could be an important mechanism whereby undesirable effects of long-term neuroglial activation can be minimized. Inflammatory mediators that are produced by activated neuroglia in CNS may have harmful effects on neurons or other neuroglial cells that they originally intended to protect [6, 7]. Thus, in various neurodegenerative diseases involving chronic neuroglial activation, neuroglial functions seem to play a more significant role in mediating diseases than in the protection of neurons. According to the model of activation-induced apoptosis of neuroglial cells, inflammatory signals that activate neuroglia may also initiate internal death program [38, 40]. One interesting question that can be raised then is how neuroglial cells could survive the inflammatory activation. It should be kept in mind that neuroglial cells *in vivo* are heterogeneous and interact with other neuroglial cells as well as neurons. There is also growing evidence that activated neuroglial cells proliferate *in vivo* as one way of replenishment [1]. Thus, not all neuroglial cells may respond to the inflammatory signals in the same fashion. Upon inflammatory activation, individual neuroglial cells in heterogeneous population may either undergo AICD or return to the resting state via other regulatory mechanisms depending on the specific microenvironment under which they react to the signals. Although many of activated neuroglial cells may be eliminated, some would survive to be deactivated. Whatever the mechanism of down-regulation is, this may be an excellent auto-regulatory system for the neuroglial activation. One can easily imagine pathological situations where this type of auto-regulatory mechanism goes wrong. Failure of the auto-regulation of ‘over-activated’ neuroglial cells may result in pathological destruction of bystander cells (neurons and other neuroglial cells) exposed to toxic me-

diators produced by activated neuroglia. Recently, up-regulated Bcl-x_L expression has been detected in reactive microglia of patients with neurodegenerative diseases [41]. Authors proposed that high level of Bcl-x_L protein might render microglia more resistant to cytotoxic environment such as areas of neurodegeneration. Expression of anti-apoptotic Bcl-2 protein has been also associated with aged brain and neurodegenerative diseases [42]. An importance of the physiological regulation of neuroglial activation by AICD is supported by these previous reports.

Recent studies focused on the possible role of neuroglia in causing neurodegeneration. Convincing evidence from *in vitro* studies pointed to the neurotoxic role of neuroglia during traumatic or ischemic brain injury [4] and AD pathogenesis [43]. Supernatants obtained from neuroglial cell cultures kill cultured neurons. Such supernatants contain various neurotoxic substances which include glutamate, NO, ROS, inflammatory cytokines, as well as yet unidentified neurotoxins [44, 45]. Production of these neurotoxins by neuroglia is enhanced by treatment with inflammatory stimuli such as lipopolysaccharide (LPS) and/or interferon (IFN)- γ . Paradoxically, other investigators have shown that neuroglia-conditioned media promote neuronal survival [46]. Thus, the balance of neurotoxic and neurotrophic effects of neuroglia appears to depend on the nature of the experimental paradigm used. In traumatic brain injury where neuronal regeneration may occur, neuroglial secretory products might help to promote regenerative efforts by injured, but surviving, neurons. However, a situation may be different in neurodegenerative diseases such as Alzheimer’s disease (AD) or human immunodeficiency virus (HIV)-associated dementia, where functionally compromised neuroglia may produce neurotoxins, thereby resulting in neuronal damage. There is considerable evidence from postmortem examinations of AD brains that auto-destructive mechanisms are at work, which could in part be responsible for the neurodegeneration [47, 48].

Neuroglia as a Target of Pharmacological Intervention

Considering neuroglial activation as a common feature in many neuropathologies, and keeping in mind that overactivation of neuroglia can have neurotoxic outcomes, it is reasonable to assume that manipulation of neuroglial activation could serve future clinical approaches [49]. Although treatment of the primary events

in neurodegenerative diseases would still be the preferred intervention, this may not always be possible. Brain or spinal cord injury is a sudden event that is followed by secondary cascades of destruction. Invading macrophages and intrinsic neuroglia in brain may carry a significant portion of these cascades of reaction. Now, there is growing evidence that toxic mediators produced by activated neuroglial cells might be involved in the pathogenesis of various neurodegenerative diseases such as Parkinson's disease (PD), AD, and HIV-associated dementia [6, 7, 47]. Thus, it is of great interest to find a means to modulate neuroglial activation and CNS inflammatory responses for the therapeutic interventions against these neurodegenerative diseases. Based on understanding of intracellular signaling pathways that are specific for activated neuroglia, a temporary inhibition of signaling molecules or protein-protein interaction associated with signaling pathways would probably allow for a rather selective effect on activated neuroglia, while respective functions in other cell types are unaffected. Elucidation of the intracellular key events that drive neuroglial activation could provide new routes for drug development [49]. Alternatively, potentially harmful products of neuroglia could be neutralized to limit undesired consequences for CNS cells and tissues. Whether it is a direct inhibition of neuroglial activation or indirect suppression of neuroglia-derived toxic inflammatory mediators, a better understanding of neuroglial biology and selective manipulation of neuroglial activation processes represent a promising goal for developing novel neuroprotective strategies.

Medical Use of Herbs

Herbs are generally defined as any form of plants or plant products, including leaves, stems, roots, and seeds [50, 51]. Herbal products may contain a single herb or combinations of several different herbs that are thought to have complementary effects. Herbal products are usually extracts of the plants, which are obtained by boiling or percolating the herbs in water, alcohol, or other organic solvents to release biologically active ingredients of the plants. Herbal products contain complicated mixtures of organic chemicals, which may include fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes and so forth. It is often difficult to determine which component(s) of the herb has biological activity. In addition, the processing of herbs, such as heating or boiling, may alter the pharmacological activity of the

organic constituents. Similarly, a host of environmental factors, including soil, altitude, and seasonal variation in weather, may affect the levels of components in any given batch of an herb. Because of these multiple factors that affect concentration of active ingredients in the final herbal product, standardization is inevitable, in which certain unique chemical components of the herbs known as markers are identified and the production process is altered to achieve a consistent level of these markers in every final batch of the herbal product. The ten most commonly used herbs in United States are echinacea, garlic, *Ginkgo biloba*, saw palmetto, ginseng, grape seed extract, green tea, St. John's wort, bilberry, and aloe [50]. The medical use of herbs in their natural and unprocessed form began when mankind first noticed that certain plants altered particular body functions. Now, much information exists about the historical use and effectiveness of botanical products. Unfortunately, however, the quality of this information is extremely variable. Necessary is evidence-based approach to the pharmacology and clinical efficacy of the herbal medicine.

Herbal Medicine against CNS Disorders

In traditional practices of Chinese medicine, numerous plants have been used to treat stroke and cognitive disorders, including neurodegenerative diseases such as AD [52, 53]. Neurodegenerative diseases and brain injuries resulting from stroke are the major and increasing public health problem in both developed and developing countries worldwide [54]. It is believed that traditional Chinese medicines (TCM) are effective, with few or no side-effects. There are more than 120 traditional medicines in use for the therapy of CNS disorders in Asian countries. Some of their therapeutic effects have been confirmed by recent clinical studies. An ethnopharmacological approach has provided a potentially rich source for drug discovery and development [55]. Many drugs currently available in Western medicine were originally isolated from plants. Although a large number of compounds have been isolated from TCMs, most of these resources have not yet been fully characterized for pharmacological purposes. Some of the TCMs used in stroke therapy include *Ledebouriella divaricata*, *Scutellaria baicalensis*, *Angelica pubescens*, *Morus alba*, *Salvia miltiorrhiza*, *Uncaria rhynchophylla*, and *Ligusticum chuanxiong* [52]. Among these, *S. baicalensis* and *U. rhynchophylla* have been found to confer neuroprotection against transient global ischemia [56, 57]. *S. baicalensis* is one of

Fig. 3. Herbal medicine as a neuroprotector that targets neuroglial inflammation. Resting neuroglia can be activated by inflammatory stimuli such as LPS, IFN- γ and TNF- α . Activated neuroglia secrete a variety of inflammatory mediators including ROS, nitric oxide, TNF- α , and IL-1 β , which cause neuronal injury (thereby resulting in neurodegeneration). Herbal medicine may be neuroprotective by blocking the inflammatory activation of neuroglia.

the most widely used herbal medicines against bacterial infections of the respiratory and gastrointestinal tract, and various inflammatory diseases. The herb has antipyretic, antibacterial, and antihypertensive properties. The main components of *S. baicalensis* – baicalin, baicalein, and wogonin – have been previously shown to exert anti-inflammatory effects [58–60]. Based on the use of *S. baicalensis* for the treatment of stroke in traditional oriental medicine, neuroprotective effects of *S. baicalensis* have been evaluated after transient global ischemia using rat 4-vessel occlusion model [56]. Methanol extracts from the dried roots of *S. baicalensis* (0.1–10 mg/kg) administered intraperitoneally significantly protected CA1 neurons against 10 min transient forebrain ischemia as demonstrated by measuring the density of neuronal cells stained with cresyl violet. The neuroprotective effects of *S. baicalensis* observed in vivo was explained in part by its inhibitory effects on microglial TNF- α and NO production as well as protection of nerve growth factor (NGF)-differentiated PC12 cells from hydrogen peroxide toxicity in vitro. *U. rhynchophylla* also exerted neuroprotective effects against transient global ischemia [57]. In traditional Oriental medicine, *U. rhynchophylla* has been used to lower blood pressure and to relieve various neurological symptoms. However, scientific evidence related to its effectiveness or precise modes of action has not been available. Methanol extract of *U. rhynchophylla* administered intraperitoneally (100–1,000 mg/kg at 0 and 90 min after reperfusion) significantly reduced the death of hippocampal CA1 neurons following the transient forebrain ischemia. Measurement of neuronal cell density in CA1 region at 7 days after ischemia by Nissl staining revealed more than 70% protection in *U. rhynchophylla*-treated rats compared to saline-treated animals. In *U. rhynchophylla*-treated animals, induction of cyclooxy-

genase-2 in hippocampus at 24 h after ischemia was significantly inhibited at both mRNA and protein levels. Furthermore, *U. rhynchophylla* extract inhibited TNF- α and NO production in BV-2 mouse microglial cells in vitro in a manner similar to what has been observed with *S. baicalensis* extract. These anti-inflammatory actions of *U. rhynchophylla* extract (and other herbal medicines) may contribute to its neuroprotective effects (fig. 3). Ginkgo leaf extracts have been primarily used for the treatment of dementia and neurosensory problems [51, 53, 61]. They contain terpenoids (ginkgolides and bilobalides) and flavonoids. Administration of ginkgo extracts (EGb 761) has shown biological activities relevant to the treatment of CNS disorders [61]. Favorable effects have been observed on cerebral circulation and neuronal cell metabolism. The extract was also neuroprotective against β -amyloid- and NO-induced toxicity [62, 63]. These effects have been attributed, in part, to platelet-activating factor antagonism of the ginkgolides [64] and the free radical scavenging and anti-oxidant properties of the flavonoids [65]. In addition to *Ginkgo biloba*, *Huperzia serrata*, *Lycoris radiata*, *Magnolia officinalis*, and *Polygonum tenuifolia* have been used for improvement of memory and cognitive function.

Plant Flavonoids as a Neuroprotector: Inhibition of Neuroinflammation

Flavonoids are a group of low molecular weight polyphenolic compounds of plant origin, many of which alter metabolic processes and have a positive impact on health [66]. They exhibit a variety of biological activities such as anti-inflammatory, anti-oxidant, anti-viral, and anti-tumor actions [67, 68]. Wogonin (5,7-dihydroxy-8-me-

Fig. 4. Neuroprotective effects of wogonin against experimental brain injury. **a–f** Treatment of experimental animals with wogonin (10 mg/kg i.p., 0 and 90 min right after 10 min ischemia and reperfusion) conferred neuroprotection by markedly reducing the number of damaged pyramidal cells in the CA1 subfield. Representative photomicrographs of cresyl violet-stained hippocampal regions of sham-operated animals (**a, d**) or animals that had been subjected to 10 min ischemia followed by treatment with either saline (**b, e**) or 10 mg/kg of wogonin (**c, f**). Boxed regions in **a, b**, and **c** ($\times 40$) are shown in **d, e**, and **f** ($\times 200$), respectively. Scale bar is 100 μ m. **g** The neuroprotective effect of wogonin was dose dependent. Either saline or wogonin (0.5, 1 and 10 mg/kg) was intraperitoneally administered into animals following 10 min ischemia. Seven days later, neuronal cell density in CA1 region was measured by Nissl

staining and cell counting. Asterisks indicate statistically significant differences from saline-treated ischemic group ($p < 0.05$). Sham = Sham-operated animals ($n = 7$); saline = saline-treated animals following ischemia ($n = 7$); wogonin = wogonin-treated animals following ischemia ($n = 3$). **h** Wogonin inhibited expression of inflammatory mediators following the ischemic brain injury. At 4 days after forebrain ischemia, the expression of iNOS and TNF- α was assessed by RT-PCR analysis of hippocampal tissue followed by Southern blot analysis using sequence-specific oligonucleotide probes. Wogonin (10 mg/kg) markedly reduced the ischemic induction of iNOS and TNF- α . Results are representative of three independent experiments. The numbers indicate a fold induction of the gene expression normalized to GAPDH as determined by densitometric analysis of Southern blot of RT-PCR products.

Table 1. Effect of flavonoid wogonin on the histological changes in hippocampus after kainate injection

Experimental animal groups	Histology scores ¹
Saline (n = 3)	0
Kainate (n = 5)	3.11 ± 0.45 ²
Kainate + wogonin, 1 mg/kg (n = 5)	2.67 ± 0.37
Kainate + wogonin, 10 mg/kg (n = 6)	2.25 ± 0.32 ²

To examine the neuroprotective effect of wogonin *in vivo*, excitotoxic neuronal injury was induced by systemic administration of kainate (30 mg/kg *i.p.*). Injection of kainate induced a severe neuronal cell death in CA1 and CA3 of hippocampus. Pretreatment with wogonin (10 mg/kg *i.p.*, 60 min prior to kainate injection) significantly attenuated the hippocampal cell death both in CA1 and CA3 as determined by histological scoring.

Values represent mean ± SEM.

¹ Damage or loss of hippocampal neurons was assessed by Nissl staining at 2 days after kainate administration with or without wogonin pretreatment. Histological damage was scored as follows: 0 = no damage; 1 = occasional injured neurons in CA1 or CA3; 2 = small area (<10%) with neuronal damage or loss in CA1 or CA3; 3 = greater area (10–50%) with neuronal damage or loss in CA1 or CA3; 4 = extended (>50%) neuronal damage or loss in both in CA1 and CA3.

² Statistically significant differences among each other ($p < 0.05$).

thoxyflavone) and baicalein (5,6,7-trihydroxyflavone) are flavonoids derived from the root of *S. baicalensis*. These flavonoids have been shown to exert various anti-inflammatory activities *in vitro* as well as *in vivo*. Wogonin inhibited LPS-induced production of NO [60, 69] and prostaglandin E₂ [70] in macrophages. Wogonin inhibited monocyte chemotactic protein-1 gene expression in human endothelial cells [71]. It also inhibited TPA-induced cyclooxygenase-2 expression and skin inflammation in mice [72]. Moreover, wogonin showed free radical scavenging and anti-oxidant activities [73–75]. In the CNS, however, little information is available about its effects on glial cells and neurons. Gao et al. [73, 76] demonstrated neuroprotective effects of four flavonoids from *S. baicalensis*, including wogonin, in cultured human neuroblastoma cells. Recently, it has been demonstrated that wogonin inhibits NO production and inducible NO synthase (iNOS) induction in cultured rat astrocytes [77], suggesting that the flavonoid may act as an anti-inflammatory agent in CNS as well. This was confirmed by a recent study where wogonin has been shown to be neuroprotective against experimental brain injury by inhibiting inflammatory activation of microglia [78]. Wogonin in-

hibited inflammatory activation of cultured brain microglia by diminishing LPS-induced TNF- α , IL-1 β , and NO production. Wogonin inhibited NO production by suppressing iNOS induction and NF- κ B activation in microglia. Inhibition of inflammatory activation of microglia by wogonin led to the reduction in microglial cytotoxicity toward co-cultured PC12 cells, supporting a neuroprotective role for wogonin *in vitro*. The neuroprotective effect of wogonin was further demonstrated *in vivo* using two experimental brain injury models; transient global ischemia by 4-vessel occlusion (fig. 4) and excitotoxic injury by systemic kainate injection (table 1). In both animal models, wogonin conferred neuroprotection by attenuating the death of hippocampal neurons, and the neuroprotective effect was associated with inhibition of the inflammatory activation of microglia. Hippocampal induction of inflammatory mediators such as iNOS and TNF- α was reduced by wogonin in the global ischemia model (fig. 4), and microglial activation was markedly down-regulated by wogonin in the kainate injection model as judged by microglia-specific isolectin B4 staining. A similar neuroprotective activity has been demonstrated with baicalein in rats [Kim et al., unpublished results] as well as in gerbils [79]. Baicalein attenuated NO production and apoptosis of LPS-activated, but not IFN- γ -activated, BV-2 mouse microglial cells as well as rat primary microglia cultures [80]. The inhibition of NO production by baicalein was due to the suppression of iNOS induction. Moreover, baicalein inhibited LPS-induced NF- κ B activity in BV-2 cells without affecting caspase-11 activation, interferon regulatory factor (IRF)-1 induction, or signal transducer and activator of transcription (STAT)-1 phosphorylation. IRF-1 and STAT-1 are central components of IFN- γ signaling [81]. Taken together, flavonoids such as wogonin and baicalein seem to exert their neuroprotective effects by inhibiting microglial activation, which is a critical component of pathogenic inflammatory responses in neurodegenerative diseases. These findings emphasize the importance of herbal medicines and their constituents as an invaluable source for the development of novel neuroprotective drugs. In neurodegenerative diseases, a pathogenic role of uncontrolled microglial activation is widely accepted at present [7]. In search of neuroprotective agents, now it is time to focus on killer cells (microglia and astrocytes) instead of killed cells (neurons); eliminating or at least suppressing killer microglial activation will provide a better chance for neuroprotection compared to just salvaging dying neurons. Identification of a potent neuroprotector from natural source that inhibits the killer cell activity

will certainly instigate further investigations in the related areas, which will ultimately lead to the successful development of novel neuroprotective drugs based on flavonoids or other constituents of the medicinal herbs.

Other Components of Herbal Medicine and Other Mechanisms of Neuroprotection

As mentioned above, herbal extracts contain complicated mixtures of organic chemicals, including fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, and terpenes. Flavonoids are not the only component possessing neuroprotective effects [82]. Anti-dementia effects of galanthamine, an alkaloid widely occurring in *Amaryllidaceous* plants, have been well demonstrated in a variety of animal models [83–85]. Acute and chronic treatment with galanthamine significantly improved the impairment of learning, short-term and spatial memory. The *p*-hydroxybenzyl alcohol and gastrodin are the active ingredients isolated from *Gastrodia elata* roots, and they have been shown to possess anti-amnesic activities in experimental animals [86–88]. Huperzine A, a sesquiterpene alkaloid purified from the Chinese medicinal herb *Huperia serrata*, exhibits a broad range of neuroprotective actions [89]. Huperzine A ameliorated learning and memory impairments and improved spatial working memory. Ginsenosides (ginseng saponins), as the major active constituents of ginseng, are another example of herb components with potent neuroprotective effects [90–92]. The cellular and molecular mechanisms underlying the neuroprotective effects of various components of herbal extracts may be as diverse as the plants which these components were isolated from. Although this review has been focused on the role of neuroinflammation in neurodegenerative diseases and its inhibition by neuroprotective herbs, the antioxidant activity of herbal extracts is certainly another important aspect of neuroprotection [93, 94]. A variety of herbal extracts and their components have been demonstrated to exert neuroprotective effects associated with antioxidant activities, either by directly stimulating antioxidant response genes or by potentiating the bodies' own natural antioxidant defense systems. Modulation of neuroinflammation and the antioxidant activity are not mutually exclusive mechanisms of action. ROS and RNS can be generated during inflammatory responses. These compounds function as important signal-transducing messengers or as an effector to kill invading microorganisms. High concentrations of ROS or RNS, however, may cause tissue injury,

which in turn leads to further inflammation. The beneficial versus detrimental effect of ROS and RNS is tightly regulated by antioxidant defense systems, whereas neuroinflammation is controlled by various endogenous mediators as well as by auto-regulatory apoptosis of inflammatory cells [30, 31]. Inflammation and generation of ROS or RNS seem to be interconnected physiological responses, which may also have pathological implications if left uncontrolled. This is supported by the findings that many herbal extracts and their components with neuroprotective activities exert both anti-inflammatory and antioxidant effects at the same time [56, 65, 95, 96].

Conclusions

Activation of microglia and astrocytes plays a pivotal role in the initiation and progression of various neurodegenerative diseases. Inhibition of the neuroglial activation may provide an effective therapeutic intervention that alleviates the progression of the neurodegenerative diseases. Herbal medicine, especially their flavonoid constituents, may be a useful candidate for such a therapeutic approach. Continual investigation of the mechanisms underlying neuroglial activation, regulation of neuroinflammation, modulatory role of herbal medicine in these processes would not only lead to the discovery of novel neuroprotective agents based on medicinal herbs, but also help to understand complex pathophysiology of neurodegenerative diseases.

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References

- Gehrmann J, Matsumoto Y, Kreutzberg GW: Microglia: Intrinsic immune effector cell of the brain. *Brain Res Brain Res Rev* 1995;20:269–287.
- Gremo F, Sogos V, Ennas MG, Meloni A, Persichini T, Colasanti M, Lauro GM: Features and functions of human microglia cells. *Adv Exp Med Biol* 1997;429:79–97.
- Stoll G, Jander S: The role of microglia and macrophages in the pathophysiology of the CNS. *Prog Neurobiol* 1999;58:233–247.
- Streit WJ, Walter SA, Pennell NA: Reactive microgliosis. *Prog Neurobiol* 1999;57:563–581.
- Kreutzberg GW: Microglia: A sensor for pathological events in the CNS. *Trends Neurosci* 1996;19:312–318.
- Minghetti L, Levi G: Microglia as effector cells in brain damage and repair: Focus on prostanooids and nitric oxide. *Prog Neurobiol* 1998;54:99–125.
- Gonzalez-Scarano F, Baltuch G: Microglia as mediators of inflammatory and degenerative diseases. *Annu Rev Neurosci* 1999;22:219–240.
- Araque A, Perea G: Glial modulation of synaptic transmission in culture. *Glia* 2004;47:241–248.
- Vesce S, Bezzi P, Volterra A: The active role of astrocytes in synaptic transmission. *Cell Mol Life Sci* 1999;56:991–1000.
- Aschner M: Astrocytes as mediators of immune and inflammatory responses in the CNS. *Neurotoxicology* 1998;19:269–281.
- Galea E, Feinstein DL, Reis DJ: Induction of calcium-independent nitric oxide synthase activity in primary rat glial cultures. *Proc Natl Acad Sci USA* 1992;89:10945–10949.
- Sawada M, Kondo N, Suzumura A, Marunouchi T: Production of tumor necrosis factor- α by microglia and astrocytes in culture. *Brain Res* 1989;491:394–397.
- Simmons ML, Murphy S: Induction of nitric oxide synthase in glial cells. *J Neurochem* 1992;59:897–905.
- Becher B, Prat A, Antel JP: Brain-immune connection: Immunoregulatory properties of CNS-resident cells. *Glia* 2000;29:293–304.
- Nakamura Y: Regulating factors for microglial activation. *Biol Pharm Bull* 2002;25:945–953.
- Ho LJ, Lai JH: Chinese herbs as immunomodulators and potential disease-modifying antirheumatic drugs in autoimmune disorders. *Curr Drug Metab* 2004;5:181–192.
- Li FQ, Lu XZ, Liang XB, Zhou HF, Xue B, Liu XY, Niu DB, Han JS, Wang XM: Triptolide, a Chinese herbal extract, protects dopaminergic neurons from inflammation-mediated damage through inhibition of microglial activation. *J Neuroimmunol* 2004;148:24–31.
- Ward PA, Warren JS, Johnson KJ: Oxygen radicals, inflammation, and tissue injury. *Free Radic Biol Med* 1988;5:403–408.
- Henson PM, Johnston RB Jr: Tissue injury in inflammation: Oxidants, proteinases, and cationic proteins. *J Clin Invest* 1987;79:669–674.
- Rankin JA: Biological mediators of acute inflammation. *AACN Clin Issues* 2004;15:3–17.
- Halliwell B: Oxygen radicals, nitric oxide and human inflammatory joint disease. *Ann Rheum Dis* 1995;54:505–510.
- Nakajima K, Kohsaka S: Microglia: Activation and their significance in the central nervous system. *J Biochem (Tokyo)* 2001;130:169–175.
- Liu B, Hong JS: Role of microglia in inflammation-mediated neurodegenerative diseases: Mechanisms and strategies for therapeutic intervention. *J Pharmacol Exp Ther* 2003;304:1–7.
- Chavarria A, Alcocer-Varela J: Is damage in central nervous system due to inflammation? *Autoimmun Rev* 2004;3:251–260.
- Popovich PG, Jones TB: Manipulating neuro-inflammatory reactions in the injured spinal cord: Back to basics. *Trends Pharmacol Sci* 2003;24:13–17.
- von Knechten A, Lotero A, Brune B: Etoposide and cisplatin induced apoptosis in activated RAW 264.7 macrophages is attenuated by cAMP-induced gene expression. *Oncogene* 1998;17:387–394.
- Albina JE, Cui S, Mateo RB, Reichner JS: Nitric oxide-mediated apoptosis in murine peritoneal macrophages. *J Immunol* 1993;150:5080–5085.
- Adler B, Adler H, Jungi TW, Peterhans E: Interferon- α primes macrophages for lipopolysaccharide-induced apoptosis. *Biochem Biophys Res Commun* 1995;215:921–927.
- Liu B, Wang K, Gao HM, Mandavilli B, Wang JY, Hong JS: Molecular consequences of activated microglia in the brain: Overactivation induces apoptosis. *J Neurochem* 2001;77:182–189.
- Lee P, Lee J, Kim S, Yagita H, Lee MS, Kim SY, Kim H, Suk K: NO as an autocrine mediator in the apoptosis of activated microglial cells: Correlation between activation and apoptosis of microglial cells. *Brain Res* 2001;892:380–385.
- Suk K, Lee J, Hur J, Kim YS, Lee MS, Cha SH, Kim SY, Kim H: Activation-induced cell death of rat astrocytes. *Brain Res* 2001;900:342–347.
- Donjerkovic D, Scott DW: Activation-induced cell death in B lymphocytes [In Process Citation]. *Cell Res* 2000;10:179–192.
- Crispe IN: Death and destruction of activated T lymphocytes. *Immunol Res* 1999;19:143–157.
- Kingham PJ, Cuzner ML, Pocock JM: Apoptotic pathways mobilized in microglia and neurons as a consequence of chromogranin A-induced microglial activation. *J Neurochem* 1999;73:538–547.
- Kingham PJ, Pocock JM: Microglial apoptosis induced by chromogranin A is mediated by mitochondrial depolarisation and the permeability transition but not by cytochrome c release. *J Neurochem* 2000;74:1452–1462.
- Yang MS, Park EJ, Sohn S, Kwon HJ, Shin WH, Pyo HK, Jin B, Choi KS, Jou I, Joe EH: Interleukin-13 and -4 induce death of activated microglia. *Glia* 2002;38:273–280.
- Takano K, Nakamura Y, Yoneda Y: Microglial cell death induced by a low concentration of polyamines. *Neuroscience* 2003;120:961–967.
- Lee J, Hur J, Lee P, Kim JY, Cho N, Lee MS, Kim SY, Kim H, Suk K: Dual role of inflammatory stimuli in activation-induced cell death of mouse microglial cells: initiation of two separate apoptotic pathways via induction of interferon regulatory factor-1 and caspase-11. *J Biol Chem* 2001;276:32956–32965.
- Lee H, Cha S, Lee MS, Cho GJ, Choi WS, Suk K: Role of antiproliferative B cell translocation gene-1 as an apoptotic sensitizer in activation-induced cell death of brain microglia. *J Immunol* 2003;171:5802–5811.
- Suk K, Kim SY, Kim H: Essential role of caspase-11 in activation-induced cell death of rat astrocytes. *J Neurochem* 2002;80:230–238.
- Drache B, Diehl GE, Beyreuther K, Perlmutter LS, König G: Bcl-xl-specific antibody labels activated microglia associated with Alzheimer's disease and other pathological states. *J Neurosci Res* 1997;47:98–108.
- Migheli A, Cavalla P, Piva R, Giordana MT, Schiffer D: bcl-2 protein expression in aged brain and neurodegenerative diseases. *Neuroreport* 1994;5:1906–1908.
- Streit WJ: Microglia and Alzheimer's disease pathogenesis. *J Neurosci Res* 2004;77:1–8.
- Giulian D: Ameboid microglia as effectors of inflammation in the central nervous system. *J Neurosci Res* 1987;18:155–171, 132–153.
- Giulian D, Haverkamp LJ, Li J, Karshin WL, Yu J, Tom D, Li X, Kirkpatrick JB: Senile plaques stimulate microglia to release a neurotoxin found in Alzheimer brain. *Neurochem Int* 1995;27:119–137.
- Nagata K, Takei N, Nakajima K, Saito H, Kohsaka S: Microglial conditioned medium promotes survival and development of cultured mesencephalic neurons from embryonic rat brain. *J Neurosci Res* 1993;34:357–363.
- McGeer PL, McGeer EG: The inflammatory response system of brain: Implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res Brain Res Rev* 1995;21:195–218.
- McGeer PL, Kawamata T, Walker DG, Akiyama H, Tooyama I, McGeer EG: Microglia in degenerative neurological disease. *Glia* 1993;7:84–92.
- Hanisch UK: Microglia as a source and target of cytokines. *Glia* 2002;40:140–155.
- Bent S, Ko R: Commonly used herbal medicines in the United States: A review. *Am J Med* 2004;116:478–485.
- De Smet PA: Herbal remedies. *N Engl J Med* 2002;347:2046–2056.
- Gong X, Sucher NJ: Stroke therapy in traditional Chinese medicine (TCM): Prospects for drug discovery and development. *Trends Pharmacol Sci* 1999;20:191–196.

- 53 Howes MJ, Houghton PJ: Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacol Biochem Behav* 2003;75:513–527.
- 54 Chalela JA, Merino JG, Warach S: Update on stroke. *Curr Opin Neurol* 2004;17:447–451.
- 55 Harvey AL: Medicines from nature: Are natural products still relevant to drug discovery? *Trends Pharmacol Sci* 1999;20:196–198.
- 56 Kim YO, Leem K, Park J, Lee P, Ahn DK, Lee BC, Park HK, Suk K, Kim SY, Kim H: Cytoprotective effect of *Scutellaria baicalensis* in CA1 hippocampal neurons of rats after global cerebral ischemia. *J Ethnopharmacol* 2001;77:183–188.
- 57 Suk K, Kim SY, Leem K, Kim YO, Park SY, Hur J, Baek J, Lee KJ, Zheng HZ, Kim H: Neuroprotection by methanol extract of *Uncaria rhynchophylla* against global cerebral ischemia in rats. *Life Sci* 2002;70:2467–2480.
- 58 Kubo M, Matsuda H, Tanaka M, Kimura Y, Okuda H, Higashino M, Tani T, Namba K, Arichi S: Studies on *Scutellariae radix*. VII. Anti-arthritis and anti-inflammatory actions of methanolic extract and flavonoid components from *Scutellariae radix*. *Chem Pharm Bull (Tokyo)* 1984;32:2724–2729.
- 59 Sekiya K, Okuda H: Selective inhibition of platelet lipooxygenase by baicalin. *Biochem Biophys Res Commun* 1982;105:1090–1095.
- 60 Wakabayashi I: Inhibitory effects of baicalin and wogonin on lipopolysaccharide-induced nitric oxide production in macrophages. *Pharmacol Toxicol* 1999;84:288–291.
- 61 Gertz HJ, Kiefer M: Review about *Ginkgo biloba* special extract EGb 761 (Ginkgo). *Curr Pharm Des* 2004;10:261–264.
- 62 Bastianetto S, Ramassamy C, Dore S, Christen Y, Poirier J, Quirion R: The *Ginkgo biloba* extract (EGb 761) protects hippocampal neurons against cell death induced by beta-amyloid. *Eur J Neurosci* 2000;12:1882–1890.
- 63 Bastianetto S, Zheng WH, Quirion R: The *Ginkgo biloba* extract (EGb 761) protects and rescues hippocampal cells against nitric oxide-induced toxicity: Involvement of its flavonoid constituents and protein kinase C. *J Neurochem* 2000;74:2268–2277.
- 64 Braquet P, Hosford D: Ethnopharmacology and the development of natural PAF antagonists as therapeutic agents. *J Ethnopharmacol* 1991;32:135–139.
- 65 Oyama Y, Chikahisa L, Ueha T, Kanemaru K, Noda K: *Ginkgo biloba* extract protects brain neurons against oxidative stress induced by hydrogen peroxide. *Brain Res* 1996;712:349–352.
- 66 Beecher GR: Overview of dietary flavonoids: Nomenclature, occurrence and intake. *J Nutr* 2003;133:3248S–3254S.
- 67 Middleton E Jr, Kandaswami C, Theoharides TC: The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev* 2000;52:673–751.
- 68 Middleton E Jr, Kandaswami C: Effects of flavonoids on immune and inflammatory cell functions. *Biochem Pharmacol* 1992;43:1167–1179.
- 69 Kim HK, Cheon BS, Kim YH, Kim SY, Kim HP: Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. *Biochem Pharmacol* 1999;58:759–765.
- 70 Wakabayashi I, Yasui K: Wogonin inhibits inducible prostaglandin E(2) production in macrophages [In Process Citation]. *Eur J Pharmacol* 2000;406:477–481.
- 71 Chang YL, Shen JJ, Wung BS, Cheng JJ, Wang DL: Chinese herbal remedy wogonin inhibits monocyte chemotactic protein-1 gene expression in human endothelial cells. *Mol Pharmacol* 2001;60:507–513.
- 72 Park BK, Heo MY, Park H, Kim HP: Inhibition of TPA-induced cyclooxygenase-2 expression and skin inflammation in mice by wogonin, a plant flavone from *Scutellaria radix*. *Eur J Pharmacol* 2001;425:153–157.
- 73 Gao Z, Huang K, Yang X, Xu H: Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Biochim Biophys Acta* 1999;1472:643–650.
- 74 Shieh DE, Liu LT, Lin CC: Antioxidant and free radical scavenging effects of baicalin, baicalin and wogonin [In Process Citation]. *Anticancer Res* 2000;20:2861–2865.
- 75 Kandaswami C, Middleton E Jr: Free radical scavenging and antioxidant activity of plant flavonoids. *Adv Exp Med Biol* 1994;366:351–376.
- 76 Gao Z, Huang K, Xu H: Protective effects of flavonoids in the roots of *Scutellaria baicalensis* Georgi against hydrogen peroxide-induced oxidative stress in HS-SY5Y cells. *Pharmacol Res* 2001;43:173–178.
- 77 Kim H, Kim YS, Kim SY, Suk K: The plant flavonoid wogonin suppresses death of activated C6 rat glial cells by inhibiting nitric oxide production. *Neurosci Lett* 2001;309:67–71.
- 78 Lee H, Kim YO, Kim SY, Kim H, Noh HS, Kang SS, Cho GJ, Choi WS, Suk K: Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. *FASEB J* 2003;17:1943–1944.
- 79 Hamada H, Hiramatsu M, Edamatsu R, Mori A: Free radical scavenging action of baicalin. *Arch Biochem Biophys* 1993;306:261–266.
- 80 Suk K, Lee H, Kang SS, Cho GJ, Choi WS: Flavonoid baicalin attenuates activation-induced cell death of brain microglia. *J Pharmacol Exp Ther* 2003;305:638–645.
- 81 Boehm U, Klamp T, Groot M, Howard JC: Cellular responses to interferon-gamma. *Annu Rev Immunol* 1997;15:749–795.
- 82 Zhang ZJ: Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Sci* 2004;75:1659–1699.
- 83 Chopin P, Briley M: Effects of four non-cholinergic cognitive enhancers in comparison with tacrine and galanthamine on scopolamine-induced amnesia in rats. *Psychopharmacology (Berl)* 1992;106:26–30.
- 84 Woodruff-Pak DS, Vogel RW 3rd, Wenk GL: Galantamine: Effect on nicotinic receptor binding, acetylcholinesterase inhibition, and learning. *Proc Natl Acad Sci USA* 2001;98:2089–2094.
- 85 Sweeney JE, Bachman ES, Coyle JT: Effects of different doses of galanthamine, a long-acting acetylcholinesterase inhibitor, on memory in mice. *Psychopharmacology (Berl)* 1990;102:191–200.
- 86 Ha JH, Shin SM, Lee SK, Kim JS, Shin US, Huh K, Kim JA, Yong CS, Lee NJ, Lee DU: In vitro effects of hydroxybenzaldehydes from *Gastrodia elata* and their analogues on GABAergic neurotransmission, and a structure-activity correlation. *Planta Med* 2001;67:877–880.
- 87 Kim HJ, Moon KD, Lee DS, Lee SH: Ethyl ether fraction of *Gastrodia elata* Blume protects amyloid beta peptide-induced cell death. *J Ethnopharmacol* 2003;84:95–98.
- 88 Kim HJ, Lee SR, Moon KD: Ether fraction of methanol extracts of *Gastrodia elata*, medicinal herb protects against neuronal cell damage after transient global ischemia in gerbils. *Phytother Res* 2003;17:909–912.
- 89 Zangara A: The psychopharmacology of huperzine A: An alkaloid with cognitive enhancing and neuroprotective properties of interest in the treatment of Alzheimer's disease. *Pharmacol Biochem Behav* 2003;75:675–686.
- 90 Wen TC, Yoshimura H, Matsuda S, Lim JH, Sakanaka M: Ginseng root prevents learning disability and neuronal loss in gerbils with 5-minute forebrain ischemia. *Acta Neuropathol (Berl)* 1996;91:15–22.
- 91 Lim JH, Wen TC, Matsuda S, Tanaka J, Maeda N, Peng H, Aburaya J, Ishihara K, Sakanaka M: Protection of ischemic hippocampal neurons by ginsenoside Rb1, a main ingredient of ginseng root. *Neurosci Res* 1997;28:191–200.
- 92 Kennedy DO, Scholey AB: Ginseng: Potential for the enhancement of cognitive performance and mood. *Pharmacol Biochem Behav* 2003;75:687–700.
- 93 Fahn S, Cohen G: The oxidant stress hypothesis in Parkinson's disease: Evidence supporting it. *Ann Neurol* 1992;32:804–812.
- 94 Metodiewa D, Koska C: Reactive oxygen species and reactive nitrogen species: Relevance to cyto(neuro)toxic events and neurologic disorders: An overview. *Neurotox Res* 2000;1:197–233.
- 95 Ahlemeyer B, Krieglstein J: Neuroprotective effects of *Ginkgo biloba* extract. *Cell Mol Life Sci* 2003;60:1779–1792.
- 96 Rahman K: Garlic and aging: New insights into an old remedy. *Ageing Res Rev* 2003;2:39–56.