Neuroprotective Effects of Fisetin by Fighting Neuroinflammation

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Abstract: *Neuroinflammation is an important event in the pathogenesis of most neurological diseases, whether acute, such as meningitis, brain trauma and stroke, or chronic, such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis. A variety of factors, such as trauma, infection, metabolic abnormalities and autoimmune diseases, etc can cause it. Neuroinflammation is characterized by the activation of astrocytes and microglia and the production of inflammatory mediators. On the one hand, neuroinflammation can protect the body, mount defenses, support tissue repair and functional recovery. On the other hand, the neuroinflammatory cascade reactions lead to blood-brain barrier breakdown and apoptosis, ultimately contributing to the progression of the disease. Therefore, the search for a drug that inhibits neuroinflammation is crucial. In recent years, fisetin has attracted much attention due to its wide range of biological activities, including anti-aging, anti-inflammatory, antioxidant and anti-cancer. In particular, the anti-inflammatory potential of fisetin has led to its increasing research in the treatment of neurological disorders. This review summarizes fisetin's therapeutic benefits and mechanisms in neuroinflammation and its potential applications.*

Keywords: Neuroinflammation; fisetin; microglia; astrocyte; anti-inflammatory

1. Introduction

Neuroinflammation is a widespread immune response of the central nervous system (CNS) caused by pathological injuries such as trauma, ischemia, autoimmune diseases, toxin accumulation and infection. Unlike peripheral inflammation, the innate immune cells involved in this process are mainly astrocytes and microglia [1]. In general, neuroinflammation helps remove dead tissue, cell debris, and pathogens. However, neuroinflammation involves the production of many factors, such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), second messenger (nitric oxide (NO) and prostaglandin, interleukin-18, interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), chemokines (CCL2, CCL5, CXCL1), and radical oxygen species (ROS) [2,3]. The continuous release of these factors creates a harsh microenvironment that exacerbates neuronal damage and is not conducive to neural repair. Unfortunately, uncontrolled neuroinflammation has instead become a key driver in the development of neurological injuries such as stroke [4], traumatic brain injury [5], and neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and Huntington's disease [6]. Recent studies have suggested that inflammation may be the root cause of cognitive problems in the brain [7]. In fact, recent ideas tend to suggest that the role of inflammation in neurodegenerative diseases such as AD may exceed the current mainstream theory of beta-amyloid (Aβ) and tau proteins [7]. Therefore, the inhibition of neuroinflammation is a crucial link in the treatment of neurological diseases. At present, through a variety of experimental models and clinical observations, researchers have applied a variety of advanced technical means, such as genomics, proteomics, transcriptomics, etc., to explore the molecular mechanism and signaling pathway of neuroinflammation. At the same time, some novel therapeutic methods have been proposed and applied, such as immunomodulators and stem cell therapy. These research results provide new ideas and directions for the diagnosis and treatment of neuroinflammation.

However, there are still some challenges and controversies in the study of neuroinflammation, such as the complexity of the disease mechanism and the differences between different types of nervous system diseases. Therefore, further in-depth research is still necessary to better understand the

occurrence and development of neuroinflammation and provide more effective methods for diagnosis and treatment of the disease. In recent years, flavonoids have attracted more and more attention due to their various effects, such as anti-oxidation, anti-tumor, anti-virus, anti-aging and anti-inflammatory [8]. Among them, fisetin is a flavonoid with potential therapeutic effects. As a natural product, fisetin has been found to reduce the inflammatory response. In addition, fisetin has also been proven to protect the heart [9], inhibit and prevent cancer [10], and prolong life [3]. In this review, we will mainly provide a detailed and specific overview of the potential of fisetin in the treatment of neuroinflammation. In addition, we also discuss the cellular mechanisms and molecular pathways for the treatment of neuroinflammation, with a view to providing new approaches for the treatment of neuroinflammation.

2. Sources, Pharmacokinetics and Toxicity of Fisetin

Fisetin is a 3, 3', 4', 7-tetrahydroxyflavone and is also a common plant pigment. It is widely found in various vegetables, fruits, and trees, such as strawberries (160 μg/g), kiwifruit (2.0 μg/g), tomatoes (0.1 μg/g), grapes (3.9 μg/g), cucumber (0.1 μg/g), onion (4.8 μg/g) and various shrubs and acacia trees [3,11,12]. In addition, it can also be obtained by chemical synthesis. The chemical structure and molecular weight of fisetin are $C_{15}H_{10}O_6$ and 286.24 g/mol, respectively. It consists of two benzene rings and one pyran ring joined together (Figure 1). The good anti-inflammatory and antioxidant effects of fisetin are mainly due to the special group in its structure. It has been found that the biological activity of fisetin depends on the double bond between C3 and C2, the carbonyl group at position 4, and the four hydroxyl substituents at position 3 ', 3, 4 ', and 7 [13,14].

Fisetin is soluble in organic solvents and almost insoluble in water. This characteristic also contributes to its poor bioavailability. Fortunately, its bioavailability can be improved through structural modifications and the development of nanomaterials, such as liposomes, alcoholsomes, polymeric micelles, glycerosomes, nanoemulsions, self-nanoemulsifying drug delivery systems, and polymeric nanoparticles [15]. Studies have shown that fisetin is metabolized mainly in the intestine and liver. The main metabolites of fisetin are geraldol, glucuronidated fisetin and glucuronidated geraldol [16]. Interestingly, fisetin is not generally detectable in systemic circulation because it rapidly undergoes metabolism to its sulphate conjugated forms and glucuronide [17]. Nevertheless, studies have shown that fisetin has a high potential for brain uptake [18] and can cross the blood-brain barrier [18-20]. This laid the foundation for its neuroprotective effect. As for toxicological studies, there have been no reports to prove that fisetin causes any toxicity.
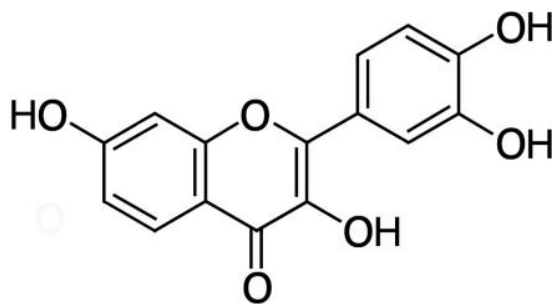

Figure 1: Chemical structure of fisetin

3. Pathophysiological Mechanism of Neuroinflammation

Neuroinflammation, as an inflammatory reaction occurring in the brain or spinal cord, has complex and diverse causes, including exogenous factors such as trauma, environmental factors and endogenous factors such as abnormal protein accumulation [21]. While the CNS responds to injury or disease by activating an inflammatory response, many neurological disorders are characterized by uncontrolled neuroinflammation. Severe neuroinflammation releases chemokines and pro-inflammatory cytokines through activated astrocytes and microglia, leading to neuronal dysfunction and disruption of the blood-brain barrier [22]. Damage to this barrier allows plasma proteins or immune cells to enter the brain parenchyma and aggravate neuroinflammation [23].

3.1. Microglia-mediated Neuroinflammation

Microglia are the innate immune cells of the CNS, involved in the development of the CNS and the maintenance of tissue homeostasis. Activation of microglia plays a key role in neuroinflammation. Studies have found that microglial activation is heterogeneous and the resulting response phenotypes can be very diverse [24]. The function and morphology of microglia are altered to adapt to changes in the microenvironment. Traditionally, it can be divided into two opposite types, the classical (M1) and the alternative (M2), which produce cytotoxic and neuroprotective effects, respectively [1]. Activation of M1 microglia secretes chemokines and pro-inflammatory cytokines, such as monocyte chemotactic protein-1, TNF-α, IL-1β and IL-6, and iNOS [25,26], ultimately exacerbating neurotoxic injury and neuropathy. In contrast, M2 microglia release anti-inflammatory mediators and growth-promoting factors such as transforming growth factor-β, interleukin-13, interleukin-10, interleukin-4, neurotrophic growth factor, insulin-like growth factor-1, colony-stimulating factor-1, and fibroblast growth factor [1,25]. It is worth mentioning that M2 microglia not only have the ability to reduce inflammation, but also promote misfolded protein degradation, neurogenesis, and neural tissue repair [26].

3.2. Astroglia-mediated Neuroinflammation

Astrocytes are the most abundant glial cells in the and play an important role in nerve support and regulation of brain homeostasis. In addition, astrocytes can also nourish nerves and regulate neuronal survival, neuronal differentiation and synaptic formation by secreting neurotrophic factors [27]. However, during inflammation, astrocytes are activated and undergo changes at the molecular and functional levels, as well as morphologically. Interestingly, reactive astrocytes can be divided into two phenotypes, type A1 and type A2, and have dual properties that hinder and support the repair of the nervous system after injury [28]. When it is activated into a neurotoxic phenotype (A1) characterized by cell hypertrophy, the production of glial fibrillary acidic protein (GFAP), complement, secretion of pro-inflammatory factors, and release of glutamate and NO increase, ultimately leading to oligodendrocyte and neuronal death [27,29]. In addition, A1 astrocytes promote the infiltration of white blood cells and increase the permeability of the blood-brain barrier, further promoting the development of the disease [27]. In contrast, A2 astrocytes up-regulate the expression of anti-inflammatory cytokines TGFβ and neurotrophic factors (such as vascular endothelial growth factor, nerve growth factor, and brain-derived neurotrophic factor) to inhibit neuroinflammation and promote neuronal generation and survival [22].

Interestingly, the activation of microglia and astrocytes is not independent of each other, but they communicate bidirectionally by releasing different signaling molecules to achieve tight mutual regulation. Studies have found that M1 microglia secrete C1q, TNF-α, IL-1β and IL-1α, which can transform astrocytes into type A1 [1,30]. A1 astrocytes secrete IL-1, CCL2, GM-CSF, CXCL10 and CX3CL1, and then activate M1 microglia [1,30]. Similarly, M2 and A2 interfere with each other. Anti-inflammatory mediators and neurotrophic factors produced by A2 astrocytes can restrict the expression of proinflammatory genes in microglia [27]. Interestingly, the decrease in inflammatory cytokines secreted by microglia also reduced the activation of A1 astrocytes.

In conclusion, the activation of astrocytes and microglia and the production of pro-inflammatory and anti-inflammatory mediators influence the outcome and development of neuroinflammation. Therefore, the regulation of these factors is of great significance for the treatment of neuroinflammation-related diseases.

4. The Role and Mechanism of Fisetin in Neuroinflammation

In recent years, the potential of fisetin as an anti-inflammatory agent has attracted the attention of many researchers. It has been found that the activation of microglia in the brain of mice and the increased ability of ATP-induced migration of BV-2 microglia can be inhibited by fisetin [31]. In addition, fisetin reduced the production of ROS, IL-1β, NO and iNOS, and upregulated the level of heme oxygenase-1 (HO-1) by activating the phosphorylation of p38 and Akt, and ultimately alleviated the motor impairment caused by lipopolysaccharide (LPS) intrabitoneal injection in mice [31]. Similarly, another study found that fisetin inhibited the production of pro-inflammatory mediators (such as TNF-α, prostaglandin E2, and COX-2) in LPS-induced primary microglia or BV-2 microglia cultures, and also reduced the cytotoxicity of LPS-stimulated microglia to neurons in co-culture systems [32]. It is worth noting that this process may act by inhibiting the phosphorylation of p38 mitogen-activated protein

kinase (MAPK), degradation of I kappa B, and nuclear translocation of NF-kappa B [32]. In addition, fisetin (20 mg/kg) has been found to inhibit LPS-induced inflammatory signaling cascade activation, phosphorylation of JNK and activation of inflammatory Toll-like Receptors 4 (TLR4)/cluster of differentiation 14 (CD14)/phospho-nuclear factor kappa B (NF-κB) signaling) [33]. As we know, Iba-1 and GFAP are markers of microglia and reactive astrocytes, respectively [34]. Interestingly, the increased expression levels of Iba-1 and GFAP proteins in the hippocampus of mice induced by LPS were decreased by fisetin, suggesting that fisetin may have potent anti-inflammatory activities by inhibiting microglial and astrocyte activation [33]. Studies have shown that during the neurotoxicity induced by AlCl3, fisetin can inhibit reactive gliosis and the expression of inflammatory cytokines in the cerebral cortex and hippocampus of mice [34]. It is worth mentioning that the treatment of fisetin can inhibit the activation of microglia induced by AlCl3, causing them to change into a branched (stationary) form [34]. In addition, it has been reported that fisetin may inhibit astrocyte proliferation and migration by reducing the phosphorylation level of Akt and extracellular signal-regulated kinase 1/2, thereby inhibiting aggressive cell phenotypes, which may lead to inhibition of glial scarring in vitro [35].

Table 1: Fisetin inhibits neuroinflammation through multiple molecular mechanisms

Types of disease/ disorder	Model	The effect of fisetin	Mechanism	Reference
Neuroinflammation	LPS/IFN-γ- or peptidoglycan-induced BV-2 microglia; H2O2-induced BV-2 microglia; ATP-induced BV-2 microglia; LPS-induced mice	ROS, IL-1β, NO and iNOS↓; HO-1↑	Phosphorylation of p38 and Akt ↑; Phosphorylation of STAT1, JAK1 and JAK2↓	[31]
Neuroinflammation	LPS-induced primary microglia or BV-2 microglia	COX-2, TNF-α, IL-1β, PGE2, NO and iNOS↓	Activation of NF-κB↓; phosphorylation of p38 MAPK↓	[32]
Neuroinflammation	LPS-induced mice	TNF-α, IL-1β, COX-2↓; Iba-1, GFAP↓	Activation of TLR4/ CD14/ NF-κB↑; phosphorylation of JNK↓	[33]
Vascular dementia	Repeated ischemia-reperfusion (IR)-induced mice	IL-1, IL-18↓	Activation of NF-κB/NLRP3 inflammasome↓	[44]
Major depressive disorder	LPS-induced mice	IL-1β, IL-6, TNF-α, iNOS↓	Activation of NF-κB↓	[36]
Intracerebral Hemorrhage	Collagenase-induced mice	TNF-α, IL-1β, IL-6↓	Activation of NF-κB↓	[37]
Cerebral ischemia-reperfusion	Transient middle cerebral artery occlusion (2 h) and reperfusion (20 h)-induced rats	IL-1, TNF-α, iNOS, IL-1β, COX-2, IL-6, PGE2, ICAM-1	Activation of NF-κB↓	[39]
Ischemic stroke	Middle cerebral artery occlusion-mice; LPS-induced N9 microglia	TNF-α↓	Activation of NF-κB↓; Phosphorylation of JNK/Jun phosphorylation↓	[38]
Aging	D-galactose-induced mice	IL-1β, Iba-1, GFAP, TNF-α, iNOS↓	Activation of p-JNK/NF-kB↓	[40]
Neuroinflammation	LPS/ATP-induced BV-2 microglia	NLRP3, ASC, caspase-1, IL-1β↓	TLR4/MD2↓; expression of MyD88 and IRAK4↓; Activation of NF-κB↓	[47]
Sepsis-associated encephalopathy	Cecal ligation and puncture operation-induced rats	IL-1R1, TNF-α, iNOS↓	Mitophagy; ↓ Activation of NF-κB/NLRP3 inflammasome↓	[48]
Neuroinflammation	Pb-induced mice	IL-6, TNF-α↓	p-AMPK, SIRT1↑; Activation of TLR4/MyD88/NF-κB↓;	[41]
Neuroinflammation	PM2.5-induced mice	IL-1β, TNF-α, IL-6, IL-8↓	Activation of NF-κB↓	[42]
Alzheimer's disease	Aβ1–42- induced mice	TNF-α, IL-1β↓	PI3K/Akt/GSK3β↑; Activation of NF-κB↓	[43]

Note: Uparrows (↑) and Downarrows (↓) represent increase and decrease, respectively. Abbreviations: IFN-γ:Interferon-gamma; ICAM-1:intercellular adhesion molecule 1; NLRP3: The NOD-, LRR-, and pyrin domain-containing protein 3; IL-1β: interleukin-1 β; NO: nitric oxide; iNOS: inducible nitric oxide synthase; HO-1: heme oxygenase-1; COX-2: cyclooxygenase-2; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; GFAP: glial fibrillary acidic protein; IL-1: interleukin-1; IL-18: interleukin-18; IL-6: interleukin-6; IL-8: interleukin-8; STAT1: Signal Transducers and Activators of Transcription 1; JAK: Janus Kinase ; NF-κB: nuclear factor kappa B; MAPK: mitogen-activated protein kinase; TLR4: Toll-like receptor 4; CD14: cluster of differentiation 14; JNK: c-Jun N-terminal kinase; MyD88: Myeloid differentiation primary response gene 88; AMPK: activators of adenosine 5'-monophosphate (AMP)-activated protein kinase; PI3K: Phosphoinositide 3-kinase; GSK3β: Glycogen synthase kinase-3 beta.

Surprisingly, fisetin preconditioning reversed LPS-induced overexpression of pro-inflammatory

cytokines (TNF-α, IL-6, and IL-1β) in the prefrontal cortex and hippocampus to improve depressive-like behavior in mice [36]. In a model of intracerebral hemorrhage, fisetin (10-90 mg/kg) treatment reduced cerebral edema, neurological severity score, and apoptosis by reducing NF-κB signaling and down-regulating pro-inflammatory cytokines [37]. It is worth mentioning that fisetin not only protected brain tissue against ischemic reperfusion injury when applied 180 minutes after ischemia but also when given 20 minutes before ischemia [38]. Similarly, fisetin inhibited the release of inflammatory cytokines in a dose-dependent manner to protect brain tissue from cerebral ischemia-reperfusion injury [39]. One experiment found that fisetin treatment (20 mg/kg/day i.p for 1 month) reduced D-gal-induced neuroinflammation in mice by reducing p-JNK/NF-kB activation [40]. Fisetin supplements reduced neuroinflammation and neurodegeneration by reversing the Pb-induced reduction in p-AMPK and SIRT1 expression levels and inhibiting the activation of TLR4/MyD88/NF-κB signaling pathway [41]. In addition, fisetin has been shown to reduce the expression of monocyte chemotactic protein-1, CXCR4, GFAP, CD11b, Iba-1, Emr-1, MIP-1α, and inhibit inflammation in the hypothalamus, hippocampus, and cortex caused by PM2.5 [42]. In the Aβ1-42-induced AD model, decreased expression of p-Akt (Ser473), p-GSK3β (Ser9), and p-PI3K was reversed by fisetin treatment [43]. At the same time, the upregulation of p-IKKβ, p-NF-kB, TNF-α and IL-1β and neurogliosis were also weakened by fisetin [44].

The NLRP3 inflammasome is a multiprotein complex that mediates caspase-1 activation and the secretion of the pro-inflammatory cytokine IL-1β/ interleukin-18 [45,46]. One study showed that fisetin mitigated cognitive deficits in mice with vascular dementia, and this protective effect may be related to its inhibition of ROS-induced activation of inflammasome components (caspase 1, ASC, and NLRP-3) [44]. Another study found that fisetin inhibited the TLR4/MD2-mediated activation of NLRP3 inflammasome and the subsequent maturation of IL-1β by eliminating damaged mitochondria in a p62-dependent manner [47]. Interestingly, fisetin blocked the activation of NLRP3 inflammasome by promoting mitochondrial autophagy in cerebral microvascular endothelial cells, reducing neuroinflammation and improving cognitive impairment [48]. Gopnar et al. suggested that neurotoxicity induced by arsenic and fluoride subacute co-exposure was reduced by fisetin, possibly due to the inhibition of TNF-α-mediated NLRP3 inflammasome [49].

In conclusion, fisetin alleviates neuroinflammation by regulating the expression of inflammatory mediators and the phenotypes of microglia and astrocytes through multiple pathways (Table 1), so as to achieve neuroprotective effects.

5. Summary and Prospect

Neuroinflammation is one of the critical factors in the occurrence and development of nervous system diseases, involving the activation of glial cells and the release of a series of cytokines. Therefore, it is of great theoretical and clinical value to study the pathophysiological process and its treatment. In recent years, it has been found that fisetin can inhibit neuroinflammation and improve nerve damage through a variety of ways, such as inhibiting the polarization of microglia, inhibiting the proliferation of astrocytes and the formation of glial scars, and reducing the production of chemokines and pro-inflammatory mediators. The main mechanisms involved include reducing the activation of NLRP3 inflammasome components and regulating MAPK, PI3K/Akt/GSK3β, TLR4/MyD88/NF-κB, JAK, SIRT1 and other pathways. The numerous anti-inflammatory benefits shown by fisetin make it a promising candidate for the treatment of neuroinflammation-related diseases. This review provides a detailed description of the beneficial effects and mechanisms of fisetin in neuroinflammation, and provides a theoretical basis for further study of clinical application of it.

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