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Research Report

Fisetin alleviates early brain injury following experimental subarachnoid hemorrhage in rats possibly by suppressing TLR 4/NF-κB signaling pathway

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ABSTRACT

Early brain injury (EBI) determines the unfavorable outcomes after subarachnoid hemorrhage (SAH). Fisetin, a natural flavonoid, has anti-inflammatory and neuroprotection properties in several brain injury models, but the role of fisetin on EBI following SAH remains unknown. Our study aimed to explore the effects of fisetin on EBI after SAH in rats. Adult male Sprague-Dawley rats were randomly divided into the sham and SAH groups, fisetin (25 mg/kg or 50 mg/kg) or equal volume of vehicle was given at 30 min after SAH. Neurological scores and brain edema were assayed. The protein expression of toll-like receptor 4 (TLR 4), p65, ZO-1 and bcl-2 was examined by Western blot. TLR 4 and p65 were also assessed by immunohistochemistry (IHC). Enzyme-linked immunosorbent assay (ELISA) was performed to detect the production of pro-inflammatory cytokines. Terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end-labeling (TUNEL) was perform to assess neural cell apoptosis. High-dose (50 mg/kg) fisetin significantly improved neurological function and reduced brain edema at both 24 h and 72 h after SAH. Remarkable reductions of TLR 4 expression and nuclear factor κB (NF-κB) translocation to nucleus were detected after fisetin treatment. In addition, fisetin significantly reduced the productions of pro-inflammatory cytokines, decreased neural cell apoptosis and increased the protein expression of ZO-1 and bcl-2. Our data provides the evidence for the first time that fisetin plays a protective role in EBI following SAH possibly by suppressing TLR 4/NF-κB mediated inflammatory pathway.

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1. Introduction

Subarchnoid hemorrhage (SAH) is a fatal subtype of stroke with more than 50% combined morbidity and mortality (Macdonald, 2014). In the past decades, studies on the pathogenesis of SAH have focused mainly on the delayed cerebral vasospasm (Laskowitz and Kolls, 2010), but the outcome is not improved even if angiographic vasospasm is reversed (Macdonald et al., 2011; Vajkoczy et al., 2005). Recent studies have shown that early brain injury (EBI), which occurs within 72 h after cerebral aneurysm rupture, seems to contribute to the poor outcome of SAH patients (Sehba et al., 2012). To improve the outcome of SAH patients, it may be a major goal to develop effective intervention on EBI treatment. The underlying mechanisms of EBI are complicated. A number of clinical and animal studies have highlighted that the inflammatory response after SAH was likely to contribute to brain injury (Prunell et al., 2005; Sehba et al., 2012). It has been reported that nuclear factor κB (NF-κB) is a predominant regulator for inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) and plays an important role after SAH (Zhou et al., 2007a). Toll-like receptors (TLRs) could lead to NF-κB activation (Barton and Medzhitov, 2002) and the expression of TLR 4 is significantly increased after experimental SAH (Zhou et al., 2007b). It has also been demonstrated that inhibiting TLR 4/NF-κB can down-regulate inflammation and be against brain injury after SAH (Ma et al., 2009; You et al., 2012). Therefore, inhibiting TLR 4/NF-κB signaling pathway may be a therapeutic target for EBI after SAH.

Fisetin (3,3′,4′,7-tetrahydroxyxflavone) is a bioactive flavonol molecule with a defined chemical structure (Fig. 1), which can be found in fruits and vegetables such as strawberry, apple, persimmon, grape, onion and cucumber at concentrations in the range of 2–160 μg/g (Arai et al., 2000). Accumulating evidence indicates that fisetin has multiple biological activities, such as antioxidant, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, antiviral (Duarte et al., 1993; Jang et al., 2005; Rice-Evans et al., 1996), neuroprotective and neurotrophic (Maher et al., 2006; Zbarsky et al., 2005). However, there is no data available about its cytotoxic and genotoxic (Lapchak, 2013). In the central nervous system (CNS), fisetin attenuats microglial neurotoxicity (Zheng et al., 2008), protects brain tissue against ischemic reperfusion injury (Gelderblom et al., 2012). However, the role of fisetin on EBI following SAH is still unknown. Thus, the aim of the present study was to determine the protective role of fisetin on EBI following SAH and whether fisetin could inhibit TLR 4/NF-κB signaling pathway in the brain.

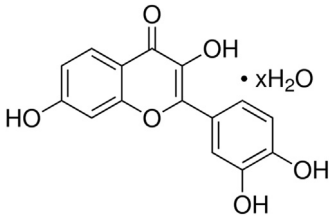

Fig. 1 – Chemical structure of fisetin(3,3′,4′,7-tetrahydroxyflavone).

2. Results

2.1. General observations and mortality rate

After SAH, there were no statistical differences observed among groups in physiological parameters (data not shown). There were 211 rats used in our study and the mortality rates after surgery were as follows: sham group 0% (0 of 42 rats), sham+fisetin 50 mg/kg group (0 of 12 rats), SAH group (7 of 49 rats), SAH+vehicle group (6 of 48 rats), SAH+fisetin 25 mg/kg group (2 of 14 rats), and SAH+fisetin 50 mg/kg group (4 of 46).

2.2. Fisetin significantly improved neurologic function and reduced brain water content at both 24 h and 72 h after SAH

Global edema is an independent risk factor for mortality and poor outcome after SAH (Claassen et al., 2002). In our study, neurologic function and brain water content were evaluated at both 24 h and 72 h after SAH. As shown in Fig. 2, neurological scores and brain water content remarkably increased after SAH compared with that in the sham group at both 24 h and 72 h (3.60 ± 0.24 vs 0.80 ± 0.20, 0.795 ± 0.001 vs 0.785 ± 0.001, $^{***}p<0.001$ and 2.00 ± 0.32 vs 0.40 ± 0.24, 0.790 ± 0.001 vs 0.785 ± 0.001, $^{*}p<0.05$) (Fig. 2). Fisetin at the dose of 25 mg/kg has no effect on neurologic function and brain edema of rats at 24 h after SAH, but 50 mg/kg of fisetin significantly alleviated neurological deficit and brain edema of rats at both 24 h and 72 h after SAH (1.80 ± 0.37 vs 3.60 ± 0.24, 0.789 ± 0.002 vs 0.795 ± 0.001, $^{\#\#}p<0.01$ and 0.60 ± 0.25 vs 2.00 ± 0.32, 0.786 ± 0.001 vs 0.790 ± 0.001, $^{\#}p<0.05$) (Fig. 2). In addition, fisetin had no effects on the sham rats. The data suggested that 50 mg/kg fisetin had neuroprotective effects on EBI after SAH.

2.3. Fisetin absolutely decreased TLR 4 protein expression in the brain cortex of rats at 24 h after SAH

To investigate the further mechanism, the levels of TLR 4 were examined. Western blot (Fig. 3A) showed that low levels of TLR 4 were expressed in the sham group. The level of TLR 4 in the SAH group was significantly increased compared with that of the sham group (1.56 ± 0.12 vs 1.13 ± 0.06, $p<0.05$) at 24 h after SAH. No significant difference was found between the SAH group and SAH+vehicle group ($p>0.05$). After fisetin (50 mg/kg) treatment, the levels of TLR 4 were significant reduced compared with that of the SAH group (1.12 ± 0.05 vs 1.56 ± 0.12, $p<0.05$). Then immunohistochemistry (IHC) (Fig. 3B) was performed to examine the immunoactivity of TLR 4. TLR 4 positive cells observed in the SAH and SAH+vehicle group (Fig. 3B) were significantly increased compared with that of the sham group (27.80 ± 2.13 vs 13.40 ± 1.007, $p<0.001$). After treatment with fisetin (50 mg/kg), TLR 4 positive cells were reduced (Fig. 3B) (17.60 ± 1.36 vs 27.80 ± 2.13, $p<0.01$).