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Research Report

Baicalein alleviates early brain injury after experimental subarachnoid hemorrhage in rats: Possible involvement of TLR4/NF-κB-mediated inflammatory pathway

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ABSTRACT

Early brain injury (EBI) following subarachnoid hemorrhage (SAH) largely contributes to unfavorable outcomes. Hence, effective therapeutic strategies targeting on EBI have recently become a major goal in the treatment of SAH patients. Baicalein is a flavonoid that has been shown to offer neuroprotection in kinds of brain injury models. This study investigated the effects of baicalein on EBI in rats following SAH. SAH was inducted in male Sprauge-Dawley rats by injection of fresh non-heparinized arterial blood into the prechiasmatic cistern. Baicalein (30 or 100 mg/kg) or vehicle were administrated 30 min after injury. Neurological deficit, brain edema, blood–brain barrier (BBB) permeability and neural cell apoptosis were assessed. To explore the further mechanisms, the change of toll-like receptor 4 (TLR4) and nuclear factor-κB (NF-κB) signaling pathway and the levels of apoptosis associated proteins were also examined. Our study showed that treatment with baicalein (30 mg/kg) significantly improved neurological function at 24 h after SAH and reduced brain edema at both 24 h and 72 h after SAH. Baicalein also significantly reduced neural cell death, BBB permeability. These changes were associated with the remarkable reductions of TLR4 expression, IκB-α degradation, NF-κB translocation to nucleus, as well as the expressions of matrix metalloproteinase-9, tight junctions protein, interleukin-1β and tumor necrosis factor- α. These findings suggest that baicalein may ameliorate EBI after SAH potentially via inhibition of inflammation-related pathway.

1. Introduction

Aneurismal subarachnoid hemorrhage (SAH) is a devastating stroke subtype with more than 50% combined morbidity and mortality (Macdonald, 2014). In the past decades, cerebral vasospasm after SAH was considered as the most important cause of high mortality and poor outcomes (Pluta et al., 2009) and has been long studied and treated, but the outcome is not

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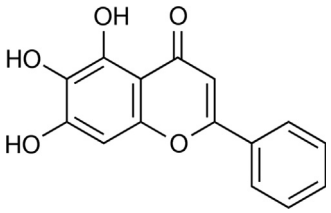

Fig. 1 – Chemical structure of baicalein.

improved even if angiographic vasospasm is reversed (Macdonald et al., 2011; Vajkoczy et al., 2005). Early brain injury (EBI) has recently been coined and refers to the immediate injury to the brain as a whole, involving brain cell death, blood –brain barrier (BBB) disruption, brain edema, and microvascular dysfunction within the first 72 h of the onset, secondary to SAH (Cahill et al., 2006), and may contribute to the high mortality and morbidity rates of SAH (Cahill et al., 2006; Macdonald, 2014). Therefore, effective EBI treatment has recently become a major goal in SAH patients care (Sehba et al., 2012). The pathophysiological mechanisms of EBI are complicated. A number of clinical and animal studies have highlighted a strong contribution of inflammation to EBI after SAH (Sehba et al., 2012). It was reported that toll-like receptors 4 (TLR4), nuclear factor-κB (NF-κB), interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α) take part in the damaging inflammatory processes after SAH. It was also reported that inhibiting TLR4/NF-κB can down-regulate inflammation and be against brain injury after SAH (Ma et al., 2009; You et al., 2012, 2013). Therefore, TLR4/NF-κB signaling pathway may be a therapeutic target for EBI after SAH.

Baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) is a flavonoid with a defined chemical structure (Fig. 1), which is isolated from the roots of Scutellaria baicalensis (Jianjun and Huiru, 2008). Evidence indicates that baicalein has multiple biological activities, like anti-inflammation (Shen et al., 2003), anti-oxidative stress (Gao et al., 2001) and inhibition of platelet aggregation (Huang et al., 2005). The neuroprotective effects of baicalein have been well documented. It has been reported that baicalein reduces inflammatory cytokines after experimental traumatic brain injury (Chen et al., 2008), attenuates cerebral cortex apoptosis (Lebeau et al., 2001) and prevents neurotoxicity induced by hydrogen peroxide (Zhang et al., 2010). All of these properties indicate that baicalein may be a potential agent for prevention and treatment of brain injury. Nevertheless, it is still unknown whether baicalein has the protective effects on EBI after SAH till now. Therefore, the present study was conducted to examine the effects of baicalein on EBI after SAH and whether baicalein can influence TLR4/NF-κB pathway in the brain after SAH.

2. Results

2.1. General observations and mortality rate

Total 151 rats were used in this study. Among them 12 rats died during the operation, which were excluded from further analysis. No statistical differences were observed among groups with regard to physiological parameters (data not shown). The mortality rates within 72 h in each group were as follows: sham group 0% (0 of 27 rats), SAH group 18.2% (6 of 33 rats), SAH+DMSO group 20.6% (7 of 34 rats), SAH+baicalein 30 mg/kg group 15.5% (5 of 32 rats), SAH+baicalein 100 mg/kg group 25.0% (2 of 8 rats).

2.2. Baicalein significantly alleviated brain edema at both 24 h and 72 h and improved neurologic function only at 24 h but not at 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, baicalein was given at two different concentrations to SAH rats. Brain edema and neurological deficit were evaluated at 24 h and 72 h after SAH. No differences were noted in the water content in cerebellum or brain stem both at 24 h and 72 h after SAH. At 24 h after SAH, treatment with baicalein at both 30 mg/kg and 100 mg/kg dramatically reduced brain water content in the cerebrum and significantly attenuated neurological deficits ($^{\#}P<0.05$, $^{\#\#}P<0.01$; Fig. 2A and C). At 72 h after SAH, baicalein (30 mg/kg) significantly reduced brain water content ($^{\#}P<0.05$; Fig. 2B) and had a tendency to reduce neurologic deficit without statistical differences ($P=0.104$; Fig. 2D). Based on these results, the optimal treatment regimen of baicalein (30 mg/kg, 30 min post-SAH) was used in the subsequent studies.

2.3. Baicalein reduced BBB permeability at 24 h after SAH

BBB permeability was tested at 24 h after SAH in our study (Fig. 3). The amount of extravasated Evans blue dye in the brain was significantly higher in the SAH group as compared to that of the sham group ($P<0.01$). The levels of extravasated dye did not differ significantly between the SAH group and SAH+DMSO groups ($P=0.907$). Baicalein treatment resulted in reduced dye extravasation into brain as compared to the SAH+DMSO group ($P<0.05$).

2.4. Baicalein reduced TLR4 protein expression at 24 h after SAH

Western blot (Fig. 4) showed that TLR4 was expressed at a low level in brains in the sham group. The level of TLR4 was significantly increased in the cortex in the SAH group as compared to that of the sham group ($P<0.01$). The protein expression did not differ significantly between the SAH group and SAH+DMSO groups ($P=0.951$). The expression of TLR4 in the brains of baicalein treatment SAH group were significantly lower than that of the SAH+DMSO group ($P<0.05$).

2.5. Baicalein blocked the degradation of IκB-α and decreased the p65 translocation to nucleus at 24 h after SAH

Next, we investigated the effects of baicalein on NF-κB activity. A decrease of total protein level of IκB-α, an indicator of NF-κB activation, was detected at 24 h after SAH. Baicalein