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Scutellarin promotes microglia-mediated astrogliosis coupled with improved behavioral function in cerebral ischemia

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ABSTRACT

Scutellarin, an anti-inflammatory agent, has been reported to suppress microglia activation. It promotes astrocytic reaction but through activated microglia. Here we sought to determine more specifically the outcomes of scutellarin treatment in reactive astrocytes in rats subjected to middle cerebral artery occlusion (MCAO). GFAP, MAP-2 and PSD-95 expression was assessed in reactive astrocytes in scutellarin injected MCAO rats. Expression of BDNF, NT-3 and IGF-1, and cell cycle markers cyclin-D1/B1 was also evaluated. *In vitro*, the above-mentioned proteins were also investigated in TNC 1 and primary astrocytes, treated respectively with conditioned medium from BV-2 microglia with or without pretreatment of scutellarin and lipopolysaccharide. Behavioral study was conducted to ascertain if scutellarin would improve the neurological functions of MCAO rats. In MCAO, reactive astrocytes in the penumbral areas were hypertrophic bearing long extending processes; expression of all the above-mentioned markers was markedly augmented. When compared to the controls, TNC1/primary astrocytes responded vigorously to conditioned medium derived from BV-2 microglia treated with scutellarin + lipopolysaccharide as shown by enhanced expression of all the above markers by Western and immunofluorescence analysis. By electron microscopy, hypertrophic TNC1 astrocytes in this group showed abundant microfilaments admixed with microtubules. In MCAO rats given scutellarin treatment, neurological scores were significantly improved coupled with a marked decrease in infarct size when compared with the matching controls. It is concluded that scutellarin is neuroprotective and that it can amplify astrogliosis but through activated microglia. Scutellarin facilitates tissue remodeling in MCAO that maybe linked to improvement of neurological functions.

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1. Introduction

It is well documented that microglia and astrocytes responded vigorously to cerebral ischemia. Indeed, microglia activation and astrogliosis are two hallmark features at the epicenter of ischemia and its penumbral region (Wang et al., 2011b). As a neuropathology sensor (Ling et al., 2001) and in response to ischemic insult, microglia undergo rapid morphological change and phagocytose cellular debris or degenerating neurons (Li et al., 2011). Activated microglia in adverse conditions are known to release a plethora of inflammatory cytokines or mediators such as TNF-α, IL-1β, NO and reactive oxygen species (Dheen et al., 2007) that may exacerbate local inflammation and neuronal damage. Microglia activation that is acute in onset is apparent as early as 1 day and becomes more pronounced between 3 and 7 days after the ischemia (Wang et al., 2011b); thereafter, it appears to subside (Yuan et al., 2014).

Along with microglia activation, cerebral ischemia can lead to

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astrocyte activation/reaction and glial scar formation (Guo et al., 2011a; Komitova et al., 2002; Raivich et al., 1999) that is crucial for sealing the site of injury and remodeling the tissue for functional restoration. Reactive astrocytes are the main constituent cells of the glial scar. In response to brain or central nervous system injuries, astrocytes exhibit specific structural and functional characteristics. Reactive astrocytes upregulate the characteristic expression of glial fibrillary acidic protein (GFAP) and various molecules such as proinflammatory cytokines or mediators (Chen and Swanson, 2003; Deng et al., 2010; Endoh et al., 1994), neurotrophic factors (Fulmer et al., 2014; Garcia-Estrada et al., 1992; Guo et al., 2011b) and nestin (Fang et al., 2015), and begin proliferating rapidly (Li et al., 2008; Pekny and Nilsson, 2005).

While both microglia activation and astrogliosis are drastically induced in cerebral ischemia following the middle cerebral artery occlusion (MCAO) in the rat, they appear to feature differentially in terms of topographical distribution and in temporal sequence. Thus, activated microglia are aggregated preferentially in the epicenter of the ischemia infarct while reactive astrocytes preponderate at the penumbral areas (Fang et al., 2015); both glial types are admixed at the "interphase" between the primary area of infarct and the outlying seemingly normal tissues. It is noteworthy that the robust microglia activation invariably precedes astrocyte reaction at 3 days which becomes more apparent at 7–14 days after MCAO (Fang et al., 2015; Yuan et al., 2014, 2015). In view of their close spatial association, it was surmised that activated microglia and reactive astrocytes at the border areas might have a paracrine relation. Indeed, it has been reported that activated microglia through production of MCSF could stimulate astrocytes in their production of proinflammatory cytokines that may be linked to chronic neuroinflammation (Deng et al., 2010). The notion of a "cross-talk" between activated microglia and reactive astrocytes was further evidenced in our recent investigation which demonstrated that activated microglia regulate astrocyte reaction in cerebral ischemia and TNC1 astrocytes *in vitro* (Fang et al., 2015). It was suggested that activated microglia and reactive astrocytes acting in concert are necessary for tissue reconstruction and remodeling for functional restoration in ischemic damage. Thus, an in-depth understanding of the involvement of microglia activation and astrogliosis either acting separately or in synchrony following cerebral ischemia is crucial to develop effective therapeutic strategies for stroke.

An inter-relationship between activated microglia and reactive astrocytes was recently put forward by us through the use of scutellarin (4,5,6-trihydroxyflavone-7-glucuronide), a major active component extracted from *Erigeron breviscapus*. Scutellarin is an herbal compound endowed with antioxidant and anti-inflammation properties (Chen et al., 2013; Hong and Liu, 2004). It has been shown to decrease microglia inflammatory response (Wang et al., 2011a). In addition to its antioxidant and anti-inflammatory properties, scutellarin has been demonstrated to have anti-apoptotic properties in animal models of ischemic stroke (Lin et al., 2007). Scutellarin significantly suppressed production of proinflammatory mediators in activated microglia in MCAO adult rats and in BV-2 microglia *in vitro* (Yuan et al., 2014, 2015). Additionally, it can regulate the expression of GFAP and nestin along with that of proinflammatory mediators in reactive astrocytes in ischemic injury (Fang et al., 2015). Remarkably, scutellarin acts to promote astrocyte reaction through activated microglia thus alluding to a "cross-talk" between the two glial cell types (Fang et al., 2015). On the other hand, the outcomes of microglia-mediated astrogliosis had remained to be fully explored. Here we extended our previous study by examining the expression of various specific biomarkers in reactive astrocytes that may be involved in differentiation, neuroprotection, migration and proliferation. The results further strengthen the inter-relationship between activated microglia and reactive astrocytes in cerebral ischemia. More specifically, we show here that scutellarin through activated microglia can promote the expression of neuronal markers, PSD-95 and MAP-2, along with enhanced expression of GFAP and neurotrophic factors including BDNF, NT-3 and IGF-1 in reactive astrocytes. It is suggested that all this would be crucial for tissue reconstruction and remodeling in cerebral ischemia for functional improvement as supported by the neurobehavioral evaluations.

2. Methods

2.1. Ethics statement on use of animals

In the handling and use of rats for middle cerebral artery occlusion (MCAO), ethical guidelines as stated in the National Institutes of Health Guide for the Care and Use of Laboratory Animals were followed. All experimental protocols and use of animals were approved by the approval authority at Kunming Medical University (KMU), and all efforts were made to minimize the number of rats used and their suffering.

2.2. Animals, surgical procedure, injection of scutellarin and animal groups

A total of 90 adult male Sprague–Dawley rats (Table 1) weighing 250–280 g were obtained from the Experimental Animal Center of Kunming Medical University. All surgical procedures were carried out in the Department of Anatomy and Histology/Embryology, KMU. The surgical procedures for MCAO followed that described previously (Wang et al., 2011b; Wu et al., 1997). Following anesthesia with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), a circular aperture of about 3 mm in diameter was created in the right parietal bone with a dental drill. The main trunk of the middle cerebral artery (MCAO) was exposed and cauterized. In the sham-operated rats, the same surgical procedure was carried out but the MCA was not cauterized. The rats were randomly divided into sham-operated + saline (sham), MCAO + saline (M), and MCAO + scutellarin (100 mg/kg) (S) groups. In the scutellarin treated group, the rats were given an intraperitoneal injection of scutellarin (100 mg/kg dissolved in saline. Cat. No. 131021, Shanghai, China) at 2 h before, and at 12, 24, 36, 48, and 60 h after MCAO. They were euthanized at 1, 3, 7 and 14 days after MCAO.

2.3. TTC assessment of infarct size

Of the 90 rats mentioned in Section 2.2, 25 rats were used for assessing the infarct size after MCAO and following scutellarin treatment using triphenyltetrazolium chloride (TTC) staining described previously by us (Yuan et al., 2014). The rats were killed at 7 and 14 d after MCAO or MCAO + scutellarin injections (n = 5 at each time points and for each group). Along with this, sham rats (n = 5) were sacrificed.

The brains were rapidly removed, frozen at −20 °C for 30 min. A total of six 2 mm thick coronal sections of the brain were then cut

Table 1
Number of rats used in various treatments.

	SHAM	MCAO (M)	S + MCAO (S)
Immunofluorescence	n = 15	n = 25	n = 25
TTC staining	n = 5	n = 10	n = 10
Total	n = 20	n = 35	n = 35

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