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Research Report

Brain recovery mediated by toll-like receptor 4 in rats after intracerebral hemorrhage

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ABSTRACT

Activation of the immune system via toll-like receptor 4 (TLR4) is implicated in both negative and positive processes in the central nervous system, including inflammation and angiogenesis. Whether TLR4 also participates in brain recovery following intracerebral hemorrhage (ICH) has not been investigated. We used the rat model of collagenase-induced ICH to determine whether TLR4 acts as a key regulator of brain recovery in the late phase of injury. After ICH, TLR4 levels in the ipsilateral striatum were significantly higher in the ICH group than in the Sham group on days 1, 3, 7 and 14 after ICH induction. By 14 d, the ICH group showed significantly higher levels of vascular endothelial growth factor, brain-derived neurotrophic factor, and MMP-9 than the Sham group, as well as greater numbers of vessels and BrdU- and DCX-positive cells. All these ICH-induced increases were significantly smaller in the TAK-242 group. The TLR4 antagonist also inhibited the recovery of neurological function after ICH. A TLR4 antagonist reduced ICH-induced neurogenesis and angiogenesis in a rat. These findings suggest that TLR4 may promote brain repair in the late phase of ICH.

1. Introduction

Intracerebral hemorrhage (ICH), which accounts for roughly 15% of all strokes, is a deadly type of stroke. Despite substantial advances in neurocritical care, more than half of patients with ICH face the prospect of death or severe disability (Liu et al., 2007; Qureshi et al., 2009). To improve the clinical outcomes of ICH, better understanding of the complex pathogenesis of ICH-induced brain injury is needed. For the same reason, more detailed insights into how physiological stimuli and pathological conditions modulate and regulate neurogenesis and angiogenesis during spontaneous brain recovery after ICH are required (Shang et al., 2011; Xiong et al., 2010; Navaratna et al., 2009).

Abbreviations: TLRs, toll-like receptors; TLR4, toll-like receptor 4; ICH, intracerebral hemorrhage; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2-deoxyuridine; MMP-9, matrix metalloproteinase-9

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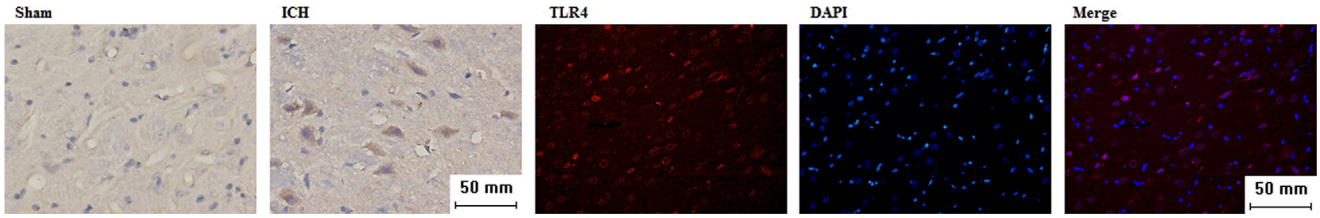

Fig. 1 – (A) Immunohistochemistry of TLR4 in sections taken from around the hematoma in rat ipsilateral striatum at 14 days. Levels of the TLR4 staining were significantly higher in the ICH group than in the Sham group at 14 day ($p<0.001$). (B Immunofluorescence double staining was used to identify the localization of TLR4 (red) relative to DAPI nuclear staining (blue). Representative results are shown ($n=5$ from each group).

One physiological signaling system that may be involved in both brain injury and brain recovery after ICH is toll-like receptors (TLRs). The TLR family comprises pattern-recognition receptors that participate in innate immunity. TLRs mediate front-line defenses in the central nervous system by recognizing exogenous and endogenous ligands that are associated with pathogens or tissue damage, such as following acute stroke (Wang et al., 2013a, 2014a, 2014b) TLRs drive both beneficial and detrimental processes in the central nervous system; which pathway they activate at a given moment depends on the brain microenvironment and on physiological and pathophysiological conditions affecting tissue homeostasis (Hanke and Kielian, 2011).

Of the various TLRs, TLR2 and TLR4 have attracted the most attention because of their involvement in cerebral injury progression. In addition to being expressed in classic immune cells, TLR4 is also expressed in neurons, astrocytes, microglia, and oligodendrocytes. TLR4 in the brain plays an important role in such detrimental processes as inflammation, multiple sclerosis, and cerebral ischemic perfusion injury, in addition to driving post-injury repair processes such as neurogenesis (Wang et al., 2013b). However, whether TLR4 helps mediate the neuro- and angiogenesis that spontaneously occur after ICH is unclear.

The cyclohexene derivative TAK-242, a small-molecule antagonist of TLR4, has been used successfully in studies of TLR4 involvement in brain injury (Matsunaga et al., 2011; Takashima et al., 2009). The molecule binds to Cys747 in the intracellular domain of TLR4, substantially reducing its activity. The molecule is quite specific, reacting exclusively with TLR4 and not with any other TLR or neuroreceptor known to be involved in brain injury or repair (Matsunaga et al., 2011; Takashima et al., 2009). Its low molecular weight and liposolubility allow TAK-242 to cross the blood–brain barrier after intraperitoneal administration (Wang et al., 2013b).

Here we examined whether TLR4 may be involved in restoring post-ICH neurogenesis and angiogenesis in a rat model of collagen-induced ICH.

2. Results

2.1. *TLR4 expression in the ipsilateral striatum*

To test whether TLR4 may be involved in ICH-induced brain repair, we measured TLR4 expression in the ICH and Sham groups at 14 days after stroke induction. Immunohistochemistry showed that TLR4 localized primarily to the cytoplasm after ICH (Fig. 1). TLR4 levels were significantly higher in ICH tissue than in Sham tissue, whereas TLR4 levels were barely detectable in Sham tissue.

Western blotting showed that TLR4 levels in the ipsilateral striatum were significantly higher in the ICH group than in the Sham group on days 1, 3, 7 and 14 after ICH induction (all $p<0.001$). Within the ICH group, levels were higher on days 3, 7 and 14 than on day 1 (ICH group on day 1 vs. ICH group on day 3, $p=0.009$; ICH group on day 1 vs. ICH group on days 7 and 14, both $p<0.001$; Fig. 2 and Table 1).

2.2. *Changes in expression of growth factors and MMP-9 in the ipsilateral striatum*

ICH induction increased the levels of VEGF, BDNF and MMP-9 in the perihematoma tissue. Western blotting showed significantly higher levels of all three proteins in the ICH group than in the Sham group at 14 days after stroke induction (all $p<0.001$; Fig. 3 and Table 1).

Administering TAK-242 after stroke led to significantly smaller ICH-induced increases in VEGF levels in the ipsilateral striatum at 14 days after stroke than in the ICH group ($p<0.001$), although the levels were still significantly higher than in the Sham group ($p<0.001$; Fig. 3 and Table 1). Similar results were obtained for BDNF, levels of which were significantly lower in the TAK-242 group than in the ICH group on day 14 ($p<0.001$), but significantly higher in the TAK-242 group than in the Sham group ($p<0.001$). Similar results were also obtained for MMP-9, levels of which were significantly lower in the TAK-242 group than in the ICH group on day 14 ($p<0.001$), but significantly higher in the TAK-242 group than in the Sham group ($p<0.001$).

2.3. *Changes in numbers of DCX- and BrdU-positive cells in the ipsilateral striatum*

Numbers of BrdU- and DCX-positive cells were significantly higher in ICH animals than in Sham animals at 14 days after ICH induction (Fig. 4). Numbers of BrdU- and DCX-positive cells in ipsilateral striatum were significantly lower in the TAK-242 group than in the ICH group at 14 days after stroke induction, though the levels in the TAK-242 group were still significantly higher than those in the Sham group (both $p<0.001$; Fig. 4).