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Research Report

Neuroprotective effect of chondroitinase ABC on primary and secondary brain injury after stroke in hypertensive rats

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ABSTRACT

Focal cerebral infarction causes secondary damage in the ipsilateral ventroposterior thalamic nucleus (VPN). Chondroitin sulfate proteoglycans (CSPGs) are a family of putative inhibitory components, and its degradation by chondroitinase ABC (ChABC) promotes post-injury neurogenesis. This study investigated the role of ChABC in the primary and secondary injury post stroke in hypertension. Renovascular hypertensive Sprague-Dawley rats underwent middle cerebral artery occlusion (MCAO), and were subjected to continuous intra-infarct infusion of ChABC (0.12 U/d for 7 days) 24 h later. Neurological function was evaluated by a modified neurologic severity score. Neurons were counted in the peri-infarct region and the ipsilateral VPN 8 and 14 days after MCAO by Nissl staining and NeuN labeling. The expressions of CSPGs, growth-associated protein-43 (GAP-43) and synaptophysin (SYN) were detected with immunofluorescence or Western blotting. The intra-infarct infusion of ChABC, by degrading accumulated CSPGs, rescued neuronal loss and increased the levels of GAP-43 and SYN in both the ipsilateral cortex and VPN, indicating enhancd neuron survival as well as augmented axonal growth and synaptic plasticity, eventually improving overall neurological function. The study demonstrated that intra-infarct ChABC infusion could salvage the brain from both primary and secondary injury by the intervention on the neuroinhibitory environment post focal cerebral infarction.

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Abbreviations: C-4-S, chondroitin-4-sulfate; ChABC, chondroitinase ABC; CNS, central nervous system; CS-GAGs, chondroitin sulfate glycosaminoglycans; CSPGs, Chondroitin sulfate proteoglycans; ECM, extracellular matrix; FITC, fluoresceine isothiocyanate; GAP-43, growth-associated protein-43; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; mNSS, modified neurologic severity scores; PBS, phosphate-buffered saline; RHRSPs, stroke-prone renovascular hypertensive rats; SBP, Systolic blood pressure; SYN, synaptophysin; VPN, ventroposterior thalamic nucleus

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1. Introduction

Stroke is a disease with morbid consequences. Not only the primary infarct, but also the secondary damages in remote loci connected to the infarction by synapses, are responsible for poor recovery after stroke (Herve et al., 2005; Schroeter et al., 2006; Villa et al., 2007). The secondary damage can be observed in the remote ventroposterior nucleus (VPN), where the delayed and selective neuronal loss displays a time profile different from that in the primary injury site (Abe et al., 2003; Loos et al., 2003; Wei et al., 2004). The VPN is a principal relaying nucleus of thalamus connected to the ipsilateral somatosensory area of the frontoparietal cortex, and both the retrograde degeneration of thalamocortical projections and the anterograde degeneration of corticothalamic tract play an important role in the secondary damage (Dihne et al., 2002; Ross and Ebner, 1990). Thus, the ipsilateral VPN is profoundly influenced by the survived tissue around the primary infarct via the synaptic connections.

Following injury in the central nervous system (CNS), there are marked glial reactions and scar formation surrounding primary injury site by reactive astrocytes, microglia and oligodendrocyte precursor cells, where the cell surface molecules and extracellular matrix (ECM) molecules produced by these glial cells can inhibit neurogenesis and thus retard the functional recovery (Bradbury and Carter, 2011; Carmichael et al., 2005; Silver and Miller, 2004). Chondroitin sulfate proteoglycans (CSPGs) are a family of the most abundant components in the ECM-rich glial scar (Bartus et al., 2012; Properzi et al., 2005; Rhodes and Fawcett, 2004), comprising a variety of core glycoproteins and covalently linked chondroitin sulfate glycosaminoglycans (CS-GAGs). During the neural development, CSPGs are involved in axonal growth and pathfinding (Bandtlow and Zimmermann, 2000; Brittis et al., 1992; Snow et al., 1990; Snow et al., 2003). In the adulthood, CSPGs are mostly present in the form of perineuronal nets at a low level, and conduct to play a role in stabilizing and restricting plasticity (Corvetti and Rossi, 2005; Galtrey and Fawcett, 2007). Among the CSPGs family, neurocan and NG2 are important members and powerful inhibitors of axonal growth and neurite extension. After neural injury, both neurocan and NG2 were up-regulated in the glial scars, impeding axonal regeneration or compensatory sprouting (Grimpe and Silver, 2002; Harris et al., 2009; Jones and Tuszynski, 2002; Jones et al., 2003). But the effect can be reversed if chondroitinase ABC (ChABC) is used to degrade CSPGs by the enzymatic removal of CS-GAGs (Bosch et al., 2012; Bradbury et al., 2002; Galtrey et al., 2007; Harris et al., 2010; Massey et al., 2006; Tester and Howland, 2008). In a recent study, ChABC treatment can reduce the thickness of glial zone and rescue neurons surrounding the cerebral infarct (Hill et al., 2012). However, it remains unknown whether the local ChABC intervention in the primary injury can protect the brain from secondary injury, which is critically important and have been suggested to predict the outcome of stroke.

The present study aimed to investigate the role of CSPGs in the primary injury of the cortex and secondary injury of the ipsilateral VPN following acute focal cortex infarction. We hypothesized that CSPGs would enhance neuronal survival and axonal growth in the ipsilateral cortex, further promote neural plasticity in the ipsilateral VPN, thus improve the overall neurological outcome.

2. Results

2.1. *The infarction was primarily located in the ipsilateral cortex sparing sub-cortex structures after middle cerebral artery occlusion (MCAO) in hypertensive rats*

Prior to bilateral renal artery clipping, the systolic blood pressure (SBP) was 99.2±6.2 mmHg in 96 rats. Twelve weeks later, stable SBP of 192.3±7.3 mmHg was achieved in all animals, while SBP in the sham group was 95.3±5.8 mmHg. The infarction was consistently induced after MCAO in stroke-prone renovascular hypertensive rats (RHRSPs), located in the ipsilateral primary and secondary somatosensory cortex sparing the thalamus, caudate putamen and midbrain (Fig. 1). There was no observed cerebral infarction in sham MCAO group.

2.2. *CSPGs were sufficiently degraded by ChABC treatment in locale*

CSPGs were barely detected in the intact adult brain, but were up-regulated in the peri-infarct area from 8 to 14 days after MCAO by immunofluorescence. After ChABC treatment, the immunoreactivity of CSPGs was significantly decreased. Accordingly, the immunoreactivity of chondroitin-4-sulfate (C-4-S), a digested product of chondroitin-4-sulfate glycosaminoglycan chains, was significantly increased over different time points (Fig. 2), demonstrating that CSPGs were sufficiently degraded in locale. However, there was no detectable positive signal of C-4-S in the ipsilateral VPN.

The full length neurocan and NG2, as the potent neuroinhibitor of main CSPGs members, were also investigated by western blotting. The levels were significantly elevated in the ipsilateral cortex at 8 and 14 days after MCAO, and were significantly reduced with intra-infarct ChABC administration compared to the vehicle ($P<0.05$, $n=6$, Fig. 3).

2.3. *ChABC treatment improved neurological function after MCAO*

Neurological function evaluated by a modified neurologic severity score (mNSS) was impaired after MCAO with a score of 9.5 (9.2, 9.8) at 1 day, 8.0 (7.6, 8.4) at 8 days, and 7.2(6.9, 7.4) at 14 days. Vehicle treatment did not make any difference on the scores over different time points. When compared with vehicle treatment, ChABC treatment ameliorated the neurological impairment with a score of 7.2(6.9, 7.4) vs. 8.3(7.7, 8.8) at 8 days and 5.6(5.2, 6.0) vs. 7.3(6.9, 7.7) at 14 days. The difference was statistically significant ($P<0.05$, $n=12$, Fig. 4).

2.4. *ChABC treatment rescued neurons in both the peri-infarct area and the ipsilateral VPN despite of identical infarct volume*

The relative infarct volumes were 17.2±2.3% at 8 days and 13.9±2.5% at 14 days after MCAO. The variation over different

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