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Research Report

# Catalpol promotes oligodendrocyte survival and oligodendrocyte progenitor differentiation via the Akt signaling pathway in rats with chronic cerebral hypoperfusion

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ABSTRACT

Chronic cerebral hypoperfusion is thought to induce white matter lesions (WMLs), which contribute to cognitive impairment. Although inflammation and oligodendrocyte apoptosis are believed to be involved in the pathogenesis of WMLs, effective therapies have not been identified yet. This study investigated whether catalpol, an iridoid glycoside, can alleviate WMLs by promoting oligodendrocyte survival and oligodendrocyte progenitor differentiation via the Akt signaling pathway in rats with chronic cerebral hypoperfusion. A rat model of chronic cerebral hypoperfusion was created through permanent occlusion of bilateral common carotid arteries. Catalpol (5 mg/kg) or saline was intraperitoneally administered daily for 10 days following the operation. On the 30th day after surgery, inflammation, oligodendrocyte apoptosis, and myelin damage in the ischemic white matter were more severe and evident than in the sham control group. Treatment with catalpol significantly suppressed white matter inflammation and attenuated oligodendrocyte apoptosis and myelin damage. The expression of phosphorylated Akt (p-Akt) and the number of mature oligodendrocytes were also markedly increased by catalpol treatment, and these effects were reversed by the PI3K inhibitor LY294002. In conclusion, catalpol attenuates hypoperfusion-induced WMLs by promoting oligodendrocyte survival and oligodendrocyte progenitor differentiation through the Akt signaling pathway. Our results suggest that catalpol may be a candidate for treating cerebrovascular WMLs.



## 1. Introduction

White matter lesions (WMLs) are commonly accompanied by neurodegenerative diseases and are responsible for cognitive impairment and gait disorders (Tomimoto et al., 2003; Burton et al., 2004). The neuropathological changes in cerebrovascular WMLs are characterized by extensive oligodendrocyte apoptosis, demyelination, and axonal injury (Tomimoto

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et al., 2003; Farkas et al., 2004); however, the underlying mechanisms leading to these changes are poorly understood. Several studies have suggested that inflammation plays an important role in provoking WMLs induced by chronic cerebral hypoperfusion (Lee et al., 2006; Duan et al., 2009), which determines the progression of neurodegenerative diseases (Narantuya et al., 2010; Wang et al., 2010). Therefore, it is important to reduce WMLs to protect against the impact of inflammatory damage. However, a treatment modality to reduce WMLs has yet to be identified.

Catalpol, an iridoid glucoside that is highly prevalent in the roots of Rehmannia glutinosa, was found to protect neurons from inflammation by inhibiting the activation of glial cells and the release of pro-inflammatory factors (Tian et al., 2006; Jiang et al., 2008). Our previous data showed that catalpol protects against oligodendrocyte apoptosis and myelin damage after chronic cerebral hypoperfusion in rats (Cai et al., 2011). The serine-threonine kinase Akt can be activated by phosphoinositide 3-kinase (PI3K)-dependent phosphorylation and has been shown to play a crucial role in promoting neuronal survival after ischemia (Yano et al., 2001; Wick et al., 2002). Catalpol has been shown to inhibit hydrogen peroxide-induced apoptosis of human endothelial cells by activating the PI3K/Akt signaling pathway (Hu et al., 2010), indicating that the anti-apoptotic effect of catalpol may be associated with Akt activation. Recently, it has been found that suppression of inflammation is a newly identified direct consequence of PI3K/Akt signaling pathway (Guha and Mackman, 2002; Yin et al., 2007). Therefore, it is crucial to investigate whether PI3K/Akt suppresses inflammation and contributes to catalpol-mediated neuroprotection of oligodendrocytes after chronic cerebral hypoperfusion.

In addition to mature oligodendrocytes, the central nervous system of adult mammals contains a small number of quiescent oligodendrocyte progenitors. Oligodendrocyte apoptosis or myelin damage can lead to rapid proliferation and migration of oligodendrocyte progenitors to demyelinating areas, where they differentiate into mature oligodendrocytes and form new myelin sheaths (Reynolds and Hardy, 1997; Redwine and Armstrong, 1998). The presence of oligodendrocyte progenitors in the adult brain may provide an opportunity for significant renewal of oligodendroglial numbers and remyelination after injury. It has been shown that catalpol increases the neurite length of forebrain neurons and dopaminergic neurons by elevating the expression of neurotrophic factors (Wang et al., 2009; Xu et al., 2010), suggesting that catalpol could promote the differentiation of neurons. However, the effect of catalpol on oligodendrocyte progenitor differentiation has not been reported.

In the present study, we induced WMLs in a rat chronic cerebral hypoperfusion model by permanent occlusion of bilateral common carotid arteries. Then, we examined whether the protective effects of catalpol on WMLs caused by cerebral ischemia were associated with its anti-inflammatory and anti-apoptotic mechanisms through activation of Akt phosphorylation. We also tested the hypothesis that catalpol promotes the differentiation of newly generated oligodendrocyte progenitors via the Akt signaling pathway.

## 2. Results

### 2.1. *Catalpol suppresses histopathologic changes of white matter*

To test whether catalpol can alleviate WMLs during cerebral hypoperfusion, we performed H&E staining to examine histopathological changes in the white matter. No signs of white matter rarefaction and vacuolation were observed in the Sha group. In the Veh group, disarrangement of nerve fibers and dark staining of the nucleus were observed. Marked white matter rarefaction, necrosis, and cavity formation were seen in the white matter including the corpus callosum. However, the above injury pattern of white matter was much less prominent in the Cat group (Fig. 1A). This effect was also reflected in the quantification of the area of vacuoles in the corpus callosum, which was significantly increased in the Veh group compared with the Sha group ($P<0.01$) and was partially reversed following treatment with catalpol ($P<0.05$) (Fig. 1C). These results verify that catalpol suppresses the histopathologic changes of white matter that are induced by chronic cerebral hypoperfusion.

Chronic cerebral hypoperfusion also caused a conspicuous increase in the number of ED1-positive cells (a marker of microglia) in the corpus callosum of the Veh group when compared with the Sha group ($P<0.01$). However, following catalpol treatment, the number of ED1-positive microglia was significantly decreased compared to the Veh group ($P<0.05$), demonstrating a reversal of the induction of microglial infiltration by catalpol (Fig. 1B and D).

To further examine whether catalpol affects the inflammatory factor TNF-α, ELISA assays were performed using homogenates from the corpus callosum of hypoperfused rats. Results showed that the content of TNF-α was much higher in the corpus callosum of the Veh group than that of the Sha group ($P<0.01$). However, in the Cat group, the content of TNF-α was significantly reduced compared with the Veh group ($P<0.05$) (Fig. 1E). This suggests that catalpol significantly suppresses the histopathological and inflammatory changes occurring in white matter due to chronic cerebral hypoperfusion.

### 2.2. *Catalpol attenuates oligodendrocyte apoptosis and myelin damage*

To determine whether catalpol suppresses oligodendrocyte apoptosis in the rat chronic cerebral hypoperfusion model, TUNEL staining was performed on brain sections. Very few TUNEL-labeled apoptotic cells were found in the Sha group throughout the corpus callosum. However, following chronic cerebral hypoperfusion, the number of apoptotic cells in the Veh group was significantly increased when compared to the Sha group ($P<0.01$), and this increase was effectively prevented by administration of catalpol ($P<0.05$) (Fig. 2A and C).

Myelin sheaths were examined by LFB staining. In the Sha group, LFB staining was clearly identified in the white matter. The normal staining pattern of LFB was markedly disrupted in the Veh group. Statistical analysis showed that the mean optical density of LFB staining in the corpus callosum