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RESEARCH ARTICLE

Buyang Huanwu decoction up-regulates *Notch1* gene expression in injured spinal cord

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Abstract

Expression of genes in the Notch signaling pathway is altered in the injured spinal cord, which indicates that *Notch* participates in repair after spinal cord injury. *Buyang Huanwu* decoction, a traditional Chinese herbal preparation, can promote the growth of nerve cells and nerve fibers; however, it is unclear whether *Buyang Huanwu* decoction affects the Notch signaling pathway in injured spinal cord. In this study, a rat model was established by injuring the T_{10} spinal cord. At 2 days after injury, rats were intragastrically administered 2 mL of 0.8 g/mL *Buyang Huanwu* decoction daily until sacrifice. Real-time reverse transcription polymerase chain reaction analysis demonstrated that at 7, 14 and 28 days after injury, the expression of *Notch1* was increased in the *Buyang Huanwu* decoction group compared with controls. These findings confirm that *Buyang Huanwu* decoction can promote the expression of *Notch1* in rats with incomplete spinal cord injury, and may indicate a mechanism to promote the repair of spinal cord injury.

***Key Words:** nerve regeneration; Buyang Huanwu decoction; spinal cord injury; Notch1 signaling pathway; Chinese medicine; neural regeneration*

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Introduction

Buyang Huanwu decoction (BYHWD) has been shown to improve blood circulation, inhibit fibroblast proliferation, and reduce liquefaction and Wallerian degeneration after spinal cord injury (SCI), as well as promote the growth of nerve cells after SCI (Cheng et al., 2001; Zhao et al., 2010; Zhang et al., 2011; Zhou et al., 2013). The mechanism underlying its promoting effect on neural regeneration may be associated with its anti-oxidative activity (Yang et al., 2011). The neuroprotective effect of BYHWD is associated with modulation of the expression of apoptosis-related proteins (Liu et al., 2014; Xian-Hui et al., 2014).

The Notch signaling pathway is highly conserved among multicellular organisms (Vaccari et al., 2008). The *Notch* gene was discovered in *Drosophila melanogaster*, and *Notch* mutations can disrupt embryonic development in *Drosophila* leading to over-differentiation of the nervous system (Poulson, 1940). We have previously shown that expression of Notch signaling pathway genes changes significantly during spinal cord repair in spinal cord-injured rats, indicating that Notch signaling might play an important role in spinal cord repair (Guo et al., 2011; Huang et al., 2012). Activation of Notch signaling is, therefore, a likely mechanism by which the locally injured micro-environment contributes to the proliferation of embryonic neural stem cells after SCI (Zhou et al., 2014). There are four Notch receptors in mammals (Notch1–Notch4), and Notch1 is localized in the cell membrane (Brou et al., 2000). This study sought to investigate the effects of BYHWD on the expression of *Notch1* so as to explore the mechanism underlying BYHWD action in the repair of SCI in a rat model of incomplete SCI.

Materials and Methods

Experimental animals

Eighty healthy, specific-pathogen-free, Sprague-Dawley adult rats weighing 150 ± 10 g, half male and half female, were provided by the Laboratory Animal Center of Liaoning Medical University in China (permission No. SCXK (Liao) 2003-2007). Rats were equally and randomly divided into normal, sham operation, SCI, and BYHWD groups. This study was approved by the Animal Ethics Committee of Liaoning Medical University, China.

Establishment of SCI models

We used a modified Allen's method (Liu et al., 2013) to generate a model of SCI: under sodium pentobarbital (40 mg/kg, intraperitoneally) anesthesia, the vertebral columns of the rats were exposed and a laminectomy at level T_{10} was carried out. A 10 g weight was allowed to fall freely from a height of 12.5 cm onto the T_{10} spinal cord both in the SCI and BYHWD groups. Rats in the SCI and BYHWD groups were treated with massage to the bladder and hind limb to

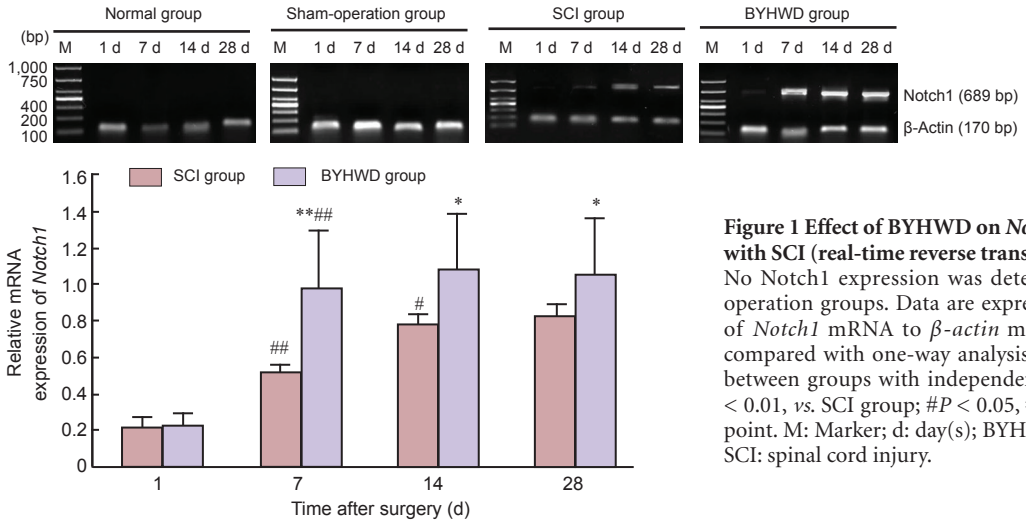

Figure 1 Effect of BYHWD on *Notch 1* mRNA expression in rats with SCI (real-time reverse transcription PCR).
No Notch1 expression was detected in the normal and sham-operation groups. Data are expressed as the optical density ratio of *Notch1* mRNA to *β-actin* mRNA (mean ± SD). Data were compared with one-way analysis of variance among groups and between groups with independent samples *t*-test.*$P < 0.05$, **$P < 0.01$, *vs.* SCI group; #$P < 0.05$, ##$P < 0.01$, *vs.* the previous time point. M: Marker; d: day(s); BYHWD: *Buyang Huanwu* decoction; SCI: spinal cord injury.

help urination. Rats that presented dysfunction in both hind limbs were considered as successful models. Rats in the sham operation group did not have their spinal cords damaged by the falling weight. Rats in the normal group were left intact.

Drug administration

BYHWD was made from angelica, astragalus, red peony root, earthworm extract, szechwan lovage rhizome, safflower and peach seed (Xian-Hui et al., 2014). The proportion of each component was 20:3:3:3:3:2:3 (Chen et al., 2008). All herbs were provided by the Department of Pharmacy, the First Affiliated Hospital of Liaoning Medical University, China. Crude preparations were decocted in water twice, and above two decoctions were concentrated to 0.8 g/mL, and then placed in cryopreservation. Rats in the normal group were given normal access to distilled water. Rats in the sham operation group and SCI group were administered distilled water intragastrically (2 mL, three times a day). Rats in the BYHWD group were administered 0.8 g/mL BYHWD by oral gavage (2 mL, three times a day). Medicine was given from day 2 after model establishment until rats were sacrificed.

Tissue treatment

At 1, 7, 14 and 28 days after surgery, five rats in each group were randomly selected and sacrificed by intraperitoneal injection of 10% chloral hydrate 5 mL/kg. Approximately 2 cm of damaged spinal cord tissue centered on T_{10} was dissected, and stored in liquid nitrogen at –80°C.

Real-time reverse transcription PCR

Total RNA in spinal cord tissue was extracted with Trizol and reverse transcription was conducted using the RNA PCR kit Ver.3.0 (TaKaRa, Dalian, Liaoning Province, China) according to the manufacturer's instructions. Primers were designed by Premier 5.0 (Sangon Biotech, Shanghai, China); *Notch1* gene: forward primer 5′-GCA GCC ACA GAA CTT ACA AAT CCA G-3′, reverse primer 5′-TAA ATG CCT CTG GAA TGT GGG TGA T-3′ (689 bp); *β-actin*: forward primer 5′-GTG GGG CGC CCC AGG CAC CA-3′, reverse primer 5′-CTT CCT TAA TGT CAC GCA CGA TTT C-3′ (170 bp). Amplification conditions were as follows: denaturation at 94°C for 5 minutes, 29 cycles of 94°C for 30 seconds, 56°C for 40 seconds and 72°C for 50 seconds, followed by extension at 72°C for 10 minutes. Reactions were preserved at 4°C. DNA products were examined by 2% agarose gel electrophoresis. Target gene expression is presented as the ratio of optical density of the gene product *versus* the internal reference.

Statistical analysis

Data are expressed as the mean ± SD and were analyzed using SPSS 16.0 statistical software (SPSS, Chicago, IL, USA). Measurement data were compared with one-way analysis of variance among groups and intergroup comparisons were made with independent samples *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

There was no significant difference in the expression of *Notch1* in rat spinal cord at different time points between normal and sham operation groups ($P > 0.05$). The expression of *Notch1* in injured spinal cord tissue increased, and then gradually stabilized in the SCI and BYHWD groups; the expression level peaked at 14 days in the SCI group and at 7 days in the BYHWD group. As expected, no significant difference in the expression level of *Notch1* in injured spinal cord was detected between the SCI and BYHWD groups on the first day. However, the expression level of *Notch1* in injured spinal cord was higher in the BYHWD group than in the SCI group at 7, 14 and 28 days ($P < 0.01$ or $P < 0.05$; **Figure 1**).

Discussion

Shin et al. (2013) found that tetramethylpyrazine (a compound in BYHWD) plays a modulatory role in microglial activation and may protect the spinal cord from, or delay, secondary SCI. Kong et al. (2014) showed that BYHWD may exert its neuroprotective effects in ischemic brain areas. Wang and Jiang (2013) confirmed that BYHWD prevented

ischemia/reperfusion-induced SCI in rats. These data indicate that BYHWD has promoting effects on rehabilitation after SCI.

The Notch signaling pathway is a key stem cell signaling pathway, and can lead to stem cell aging (Sun et al., 2008). Homozygous gene disruption of Notch1 and Notch2 in mice causes embryonic death (Okuhashi et al., 2013). Zhou et al. (2014) showed that activation of the Notch signaling pathway was one of the mechanisms by which the local injured microenvironment contributes to the proliferation of embryonic neural stem cells after SCI, and it may involve up-regulation of *Notch1* and *Hes1* expression.

The inactivation of *Notch1* in neural precursor cells in mice leads to neural cell differentiation, which is consistent with a prior study on *Notch1* gene knockout (Chang et al., 2013; Piccin et al., 2013). Homozygous ablation of Notch1 and Notch2 causes embryonic death in mice (Dijkers and Faotto, 2012). The inactivation of *Notch1* in neural precursor cells causes their differentiation into nerve cells (Wegener et al., 2012). In this study, the expression of *Notch1* in rats after SCI was higher in the BYHWD group compared with that in the SCI group at 7, 14 and 28 days. Compared with the SCI group, the BYHWD group reached the maximal expression of *Notch1* gene earlier, and the high level of expression continued for longer. These results suggest that the Notch pathway plays an important role in BYHWD-stimulated repair.

In conclusion, BYHWD affected the microenvironment and up-regulated *Notch1* gene expression in rats after SCI. Thus, BYHWD promoted the repair of SCI. However, due to the myriad factors influencing SCI repair and the complex composition of BYHWD, the working mechanism of BYHWD action cannot be explained at present and deserves further investigation.

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Conflicts of interest: *None declared.*

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