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The effect of simvastatin treatment on proliferation and differentiation of neural stem cells after traumatic brain injury

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ABSTRACT

Objective: To study the effect of simvastatin on neurological functional recovery after traumatic brain injuries (TBI) and the possible molecular mechanisms, we evaluated simvastatin-induced proliferation and differentiation of neural stem cells (NSCs) in vitro and in vivo and possible involvement of Notch-1 signaling in this process.

Methods: Adult Wistar rats were randomly divided into three groups ($n=28$ for each): sham group, saline-treated group and simvastatin-treated group. Simvastatin was given orally at a dose of 1 mg/kg/day starting at day 1 after TBI. At 1, 3, 7, 14, 21, 28, and 35 days after simvastatin treatment, functional outcome was measured using modified neurological severity scores (mNSS). Immunofluorescence of nestin was used to identify neurogenesis of NSCs in injured area of TBI rats. Western blot was applied to detect the expression level of Notch-1 protein in TBI rats with simvastatin.

Results: Immunostaining showed a significant increase in the number of nestin-positive cells in injured area of the simvastatin-treated group compared to that of the saline-treated group ($p<0.05$). In in vitro experiment, simvastatin induced enhanced proliferation and neurogenesis of cultured NSCs and elevated Notch-1 protein expression. Co-incubation of γ-secretase inhibitor, an inhibitor of Notch-1 pathway, with simvastatin abolished its neurorestoration effect. Most importantly, the simvastatin-treated group had

Abbreviations: NSCs, neural stem cells; TBI, traumatic brain injury; mNSS, modified neurological severity scores; NICD, Notch intracellular domain; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; MAP-2, microtubule-associated protein 2; DMEM, dulbecco's modified eagle medium; 3H-TdR, tritiated thymidine; DG, dentate gyrus; DAB, diaminobenzidine

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significantly decreased mNSS at day 35 after TBI compared with the saline-treated group ($p < 0.05$).

Conclusion: Simvastatin treatment enhanced neurological functional recovery after TBI possibly via activation of Notch signaling and increasing neurogenesis in the injured area.

1. Introduction

TBI is a major health and socio-economic problem that affects global society. However, therapeutic option for the treatment is still limited. Simvastatin has been shown to exert beneficial effects on several models of neurotrauma and it might be one potential treatment for patients with TBI. In animal models of TBI, simvastatin treatments have been shown multiple functions including long-lasting functional improvement (Mahmood et al., 2009), promoting the proliferation of endothelial cells, increasing the length of vascular perimeter (Wu et al., 2011), preventing brain trauma-induced increase in beta-amyloid peptide level (Abrahamson et al., 2009), and reducing inflammatory cytokine released from astrocytes (Li et al., 2009). Simvastatin has also been reported to suppress the phosphorylation of epidermal growth factor receptor in lipid rafts of astrocytes after oxygen-glucose deprivation in vitro (Wu et al.,2009). After TBI, simvastatin was reported to suppress apoptosis and upregulation of VEGF and BDNF through activation of Akt signaling pathway (Wu et al., 2008). All these studies suggest a neuroprotective role of simvastatin-treatment after brain injury.

Although neuroprotective effects of simvastatin have been reported, the underlying molecular and cellular mechanisms remain unclear. It has been suggested that simvastatin enhanced neuronal survival in hippocampal CA3 region and increased neurogenesis in dentate gyrus (Lu et al., 2007). However, little is known about the effect of simvastatin on neurogenesis in the brain with TBI. It is also not clear whether simvastatin has similar effect on neurogenic and non-neurogenic region after TBI. The Notch signaling pathway, which plays a key role in multiple cell functions such as differentiation, proliferation, and apoptosis, has been shown to be regulated by simvastatin (Xu et al., 2009; Zacharek et al., 2009). Simvastatin-induced activation of Notch can be inhibited by a gamma-secretase inhibitor, which blocks Notch signaling activity and prevents Notch intracellular domain (NICD) production.

In this study, we explored the effect of simvastatin on proliferation and differentiation of NSCs in injured region after TBI and asked whether Notch-1 signaling pathway mediated simvastatin-induced effect.

2. Results

2.1. Simvastatin increased nestin-positive cells

To investigate the effect of simvastatin on proliferation of NSCs in injured area, we used immunostaining for nestin, a protein marker for NSCs in adult brain. Simvastatin-treated group (Fig. 1A) showed a significant increase in nestin-positive cells in the lesion boundary zone in brains with TBI compared with saline-treated (Fig. 1A), or sham group (Fig. 1A) at all evaluated time-point (Fig. 1B). Increased nestin-positive cells surrounding lesion area suggested increased neurogenesis after TBI. This result indicated that the beneficial outcome of simvastatin treatment might be related to increased neurogenesis in injured area.

2.2. γ-Secretase inhibitor abolished the effect of simvastatin on NSCs proliferation

It has been reported previously that simvastatin induced expression of Notch-1 protein during NSC proliferation (Zacharek et al., 2009). Gamma-secretase inhibitor has been shown to inhibit Notch signaling pathway in cells. To explore the possible involvement of Notch signaling pathway in simvastatin-induced neurogenesis in injured area after TBI, we used γ-secretase inhibitor in our experiment. The γ-secretase inhibitor (1 μmol/L) was applied in the presence of 1.25 μmol/L simvastatin in cultured NSCs. NSCs were plated in growth medium until formation of small clusters and then larger floating neurospheres (Fig. 2A). Immunostaining of sectioned neurospheres revealed that these cells were nestin-positive (Fig. 2B). The 3 *H*-thymidine incorporation assay, in which 3*H*-thymidine was incorporated into new strands of chromosomal DNA during mitotic cell division, was used to evaluate the proliferation of NSCs in the presence of simvastatin (Fig. 2C). The nestin-positive cells co-labeled with 3*H*-thymidine were presented in Fig. 2D. NSCs with 3*H*-thymidine incorporation were collected and CMP was measured for comparison of NSCs proliferation among different groups (Fig. 2E). Compared with DMSO, 1.25 μmol/L simvastatin significantly increased proliferation of NSCs in culture, showing as a significant increase in CMP value. Application of 1 μmol/L γ-secretase inhibitor significantly reduced simvastatin-mediated proliferation of NSCs. This result suggested that inhibiting Notch signaling pathway abolished the effect of simvastatin on NSCs proliferation.

2.3. γ-Secretase inhibitor abolished simvastatin-induced Notch-1 expression

We further used WB to detect level of Notch-1 expression in NSCs in response to simvastatin and/or γ-secretase inhibitor administration. Treatment with 1.25 μmol/L simvastatin increased level of Notch-1 expression in NSCs and it was reversed by administration of 1.0 μmol/L γ-secretase inhibitor (Fig. 3). This result indicated that simvastatin-induced