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VEGF inhibits the inflammation in spinal cord injury through activation of autophagy

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ABSTRACT

Vascular endothelial growth factor (VEGF) is a secreted mitogen associated with angiogenesis and re-vascularization of spinal cord injury (SCI). VEGF has long been thought to be a potent neurotrophic factor for the survival of spinal cord neuron. However, the neuroprotective mechanism of VEGF is still unclear. The aim of this study was to investigate the effect of VEGF on spinal cord injury and its mechanisms. Young male Wistar rats were subjected to SCI and then $VEGF_{165}$ were injected directly into the lesion epicenter 24 h post injury. We detected Basso, Beattie and Bresnahan (BBB) scores and numbers of motor neuron via Nissl staining. The expressions of autophagy related protein Beclin1 and LC3B were determined by Western blot and RT-PCR. We also detected the contents of inflammation factors interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α) and interleukin-10(IL-10) in LPS (Lipopolysaccharide) treated spinal neuron-glia co-culture by ELISA. We found that $VEGF_{165}$ administration increased the BBB score and reduced the loss of motor neuron of rats induced by SCI. VEGF decreased the protein expressions of IL-1β, TNF-α and IL-10 and up-regulated the expressions of Beclin1 and LC3B of rats. In the *in vitro* study, $VEGF_{165}$ decreased the levels of IL-1β, IL-10 and TNF-a in the medium of LPS treated spinal neuron-glia co-culture, which was partially blocked by 3-MA, the inhibitor of autophagy. In addition, $VEGF_{165}$ up-regulate the expressions of Beclin1 and LC3B in co-culture cells. The results suggested that $VEGF_{165}$ attenuated the spinal cord injury by inhibiting the inflammation and increasing the autophagy function.

1. Introduction

The pathophysiology of acute spinal cord injury (SCI) involves primary and secondary mechanisms of injury [1]. Secondary injury mechanisms include ischemia, vascular changes, electrolyte disorders, inflammation, edema and loss of energy metabolism [2]. The secondary inflammation and vascular disruption of spinal cord tissue after SCI was critical for the survival of motor neuron and functional recovery [3].

Vascular endothelial growth factor (VEGF) is a secreted mitogen associated with angiogenesis by binding to the VEGFR1 or VEGFR2 [4]. The VEGF isoforms such as 121,165,189 were detected in human and 120,164,188 in mice. Although VEGF isoforms play important roles in the development of vascular network, it independently works on neuron to guide neurogenesis [5], axon growth [6–8], synaptic plasticity and dendrite patterning [9,10]. VEGF defense motoneurons from insults free radicals [11], hypoxia/hypoglycemia [12], glutamate-excitotoxicity [13,14] and thus plays important role in the recovery of SCI.

Autophagy is activated to clear cytoplasmic components and to stabilize the microenvironment by sequestrating and digesting in autophagosomes after SCI. Autophagy protects neurons from degradation and inhibiting autophagy advanced neurodegeneration [15]. Autophagy protects neurons from infection and degradation which may arbiter neuron cells death or survival [16]. The present study was to observe the effect of VEGF on spinal cord injury. We found that VEGF attenuated the spinal cord injury by inhibiting the inflammation and increasing the autophagy function.

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2. Methods and materials

2.1. Acute spinal cord injury model

Adult male Sprague–Dawley rats (250–300 g) were purchased from Capital Medical University (Beijing, China). All animals were housed in standard temperature conditions with a 12 h light/dark cycle and regularly fed with food and water. Following 10% chloric hydras (3 ml/kg, i.p.) anesthesia, rats were positioned on a cork platform. Incision the skin to expose the vertebral column and to perform a laminectomy carried out at the T9 level. A contusion was induced by a self-made weight-drop device in the spinal cord corresponding to the T9 spinous process, centering at the posterior median spinal vessels. The striking force was 25 × 3 g*cm: the iron stick was 25 g in weight and 3 cm in bottom diameter, the dropping distance was 3 cm, and the time of contact with the dura mater was 0.1 s. During the surgery, the body temperature was maintained at 36.0–37.0 °C by incandescent lamp exposure. After injury, the wound was closed.

2.2. Neuron/glial co-cultures

The co-culture mixed glia cells (astrocyte and microglia) were extracted from spinal cords of Sprague–Dawley rat pups(<48 h), the tissue was isolated under the microscope and soaked in 0.1 M phosphate-buffered saline (PBS) (pH 7.4) with 0.6% glucose. Co-cultured cells were played on poly-DL-lysine (30–70 kDa, Sigma–Aldrich, St. Louis, MO, USA) coated glass coverslips of 24-wells at a density of 5×10^4 cells/well in high glucose Minimum Essential Medium (MEM) containing GlutaMaxTM supplemented with 10% fetal bovine serum, 25 mg/ml penicillin/streptomycin (GIBCO). The cells were cultured for a week at 37 °C and exchange medium regularly. Then, embryos were removed from pregnant rats on the 17th or 18th gestational day by cesarian section. Cells were dissociated from embryo spinal cords as described above. Neurons (2.5×10^4/well) were plated on astrocyte/microglia monolayer and the cultures were maintained with regular MEM. All cells were grown at 37 °C and in 5% CO_2. Most of the experiments were performed on 16–20 days *in vitro* cultures.

2.3. Drug administration

The animals were randomly assigned into 3 groups (5 rats per group). Sham group: rats received laminotomy alone; SCI group: rats received SCI and micro-injection of 1.5 μl of 1 × PBS; VEGF group: rats received SCI and micro-injection of human recombination $VEGF_{165}$ (4ug/ml; R&D Systems) in 1.5 μl of 1 × PBS into the spinal cord at 24 h post injury. Drugs were injected right at the injured site 1.2 mm into the spinal cord at the rate of 0.5ul/min. Drugs were given for continuous three days.

Neuron-glia co-culture cells were treated with 100 ng/ml of VEGF and 3-MA (5uM) 4 h prior to stimulation with LPS (100 ng/ml) , at 24 h later, supernatants in the medium were collected for detection.

2.4. Nissl staining

Spinal cord slices were incubated at 60 °C for 30 min, then cooled, cleared with xylene for 2 × 5 min, dehydrated with 100%, 95%, 90%, 85% ethanol each for 5 min, with 80%, and 70% ethanol each for 3 min. Then slices were rinsed with distilled water for 1 min, stained with cresyl violet dye in an oven at 37 °C for 30 min, rinsed with water for 8 min, and rapidly separated with 95% ethanol. Then sections were incubated in anhydrous alcohol and xylene for 2 × 5 min. Slices were mounted with neutral gum.

2.5. Western blot and ELISA analysis

Co-cultured cells and spinal cords encompassing the injury site were collected and lysed with RIPA buffer with PMSF (Beyotime Biotechnology, China). The lysates were centrifuged at 12,000 g for 20 min at 4 °C. The samples were separated by SDS–PAGE and transferred to the PVDF membranes. After incubating with primary antibodies overnight at 4 °C and secondary antibodies for 1 h at room temperature, the bands were visualized by an ECL detection system (Pierce Chemical, Rockford, IL, USA) and quantified by Image J software (NIH, Bethesda, MD). Primary antibodies used are as follows: Beclin1 (1:1000; rabbit IgG, 1:1000; Cell Signaling, Danvers, MA.); LC3B (rabbit IgG, 1:1000; Abcam, Cambridge, UK.); β-actin (rabbit IgG, 1:2000; Santa Cruz Biotechnology, Santa Cruz, CA).

After 24 h in the co-cultures, 20 μl conditioned medium was harvested for IL-1β, IL-10, TNF-α were measured using respective ELISA kit according to the manufacturer's instructions and analyzed by microplate reader (Dynex Technology, Chantilly, VA, USA).

2.6. RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) analysis

Spinal cord tissues were collected from rats in each group, total mRNA were extracted from the injury sites using the Trizol reagent protocol (TianGen), and Beclin1 and LC3B expression was investigated using the OneStep RT-PCR kit according to the manufacturer's protocol. β-actin was used as an internal reference cDNA synthesis was carried out at 50 °C for 30 min, and the PCR conditions were as follows: 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min and one cycle of final extension. The primers used were as follows: Beclin1 forward: 5′-GAA CCG CAA GAT AGT GGC-3′, reverse: 5′-CAG AGC ATG GAG CAG CAA-3′; LC3B forward: 5′-GAG CAG CAT CCA ACC AAA-3′, reverse: 5′-CGT CTC CTG GAG GCA TA-3′ and β-actin forward: 5′-AGCTACGAGCTGCCTGACG-3′, reverse: 5′-GCATTTGCGGTGGACGAT-3′; PCR products were separated by gel electrophoresis and visualized by staining with ethidium bromide and visualized under UV light.

2.7. Immunofluorescence Staining

Cells were fixed with 3.7% formalin containing 0.1% Triton X-100 in PBS for 10 min at room temperature. The coverslips were incubated with blocking buffer (PBS containing 5% goat serum, 3% bovine serum albumin, 0.1% Triton X-100) for 30 min at room temperature. Primary antibodies were diluted in blocking buffer and incubated with the cells at room temperature for 2 h. Primary antibody used in this study was: anti-LC3B (Rabbit IgG, 1:500; Abcam, Cambridge, UK.). Cells were washed for 4 × 10 min at room temperature and then incubated with Alexa Fluor 594/647 FITC goat-anti-rabbit secondary antibody at a dilution of 1: 400 for 2 hat room temperature. The glasses were rinsed three times with PBS and incubated with medium containing DAPI (Vector Laboratories) for 15 minutes to counterstain the nuclei. All images were captured on Leica DMI4000B microscope (Leica Microsystems, Wetzlar, Germany).

2.8. Statistical analysis

Data are expressed as the mean ± SEM by SPSS17.0. Two experimental groups were determined by Student's t-test. When more than two groups were compared, one-way analysis of variance (ANOVA) and Dunnett's post hoctest was used to evaluate the data. P values <0.05 were considered statistically significant.

3. Results

3.1. VEGF promotes functional neurobehavioral recovery after SCI

All rats had normal limb function with a BBB score of 22 before SCI. There was no locomotor dysfunction in rats from sham surgery group with a BBB score of 22 throughout the study. Serious hind limb locomotor dysfunction (complete paralysis) was found in rats after SCI alone, which was significantly improved by VEGF treatment ($p < 0.05$) at day 1, day 7, and day 21 post SCI (Fig. 1A). The results suggested that VEGF improves the dysfunction caused by SCI.

3.2. VEGF decreases motor neuron loss and reduces cavity area

The effect of VEGF on motor neurons in SCI was investigated using Nissl staining at day 7 after contusion. SCI rats showed great loss of large anterior horn cells compared with the Sham group. VEGF significantly preserved motor neurons in the anterior horns of rats (Fig. 1B, C and D). VEGF treatment significantly improved numbers of motor neuron compared with SCI group (Fig. 1E). Spinal cords from VEGF treated rats exhibited a greater extent of spared tissue up to 2 mm rostral and caudal to the injury epicenter (Fig. 1F). This experiment revealed an overall significant improvement in tissue preservation in the VEGF treated group.

3.3. VEGF attenuates inflammation response and up-regulates Beclin1 and LC3B in SCI rats

VEGF administration decreased the expression of inflammation factors IL-1β, IL-10 and TNF-α at 7D after SCI by Western blot (Fig. 2A) ($p < 0.05$). We also detected autophagy related protein Beclin1 which initiates the formation of autophagosomes and the microtubule-associated protein light chain 3B (LC3B), one of the main autophagy marker. Protein and mRNA expressions of Beclin1 were significantly higher in SCI group than Sham group ($P < 0.05$), which was further increased by VEGF treatment ($P < 0.05$). LC3B expression was higher in SCI group than Sham group at levels of protein and mRNA, which was enhanced after VEGF treatment ($P < 0.05$) (Fig. 2E, H).

3.4. VEGF reduces the levels of cytokines in spinal neuron-glia co-culture treated by LPS

To confirm its protective effect, we tested the effect of VEGF on primary neuron-glia co-cultured response to LPS exposure. The protein contents of IL-1β, TNF-α and IL-10 were significantly increased in the medium of neuron-glia co-culture treated with LPS. VEGF significantly reduced the contents of IL-1β, TNF-α and IL-10 compared with LPS group, which was partially blocked by 3-MA (Fig. 3A, B and C).

3.5. VEGF up-regulates the expression of Beclin1 and LC3B of neuron-glia co-cultured cells treated by LPS

The results showed that protein expressions of Beclin1 in neuron-glia co-culture were significantly higher in LPS group than control group ($P < 0.05$), which was further increased by VEGF treatment ($P < 0.05$). LC3B expression was higher in LPS group than control group, which was enhanced after VEGF treatment ($P < 0.05$) (Fig. 4A). Immunofluorescence staining showed that VEGF treatment significantly increased expressions of LC3B at 1D in neuron-glia co-cultured cells in LPS induced toxicity (Fig. 4D).

4. Discussion

Large amount of studies have revealed that administration of VEGF to the injured spinal cord has neuroprotective effects, induces

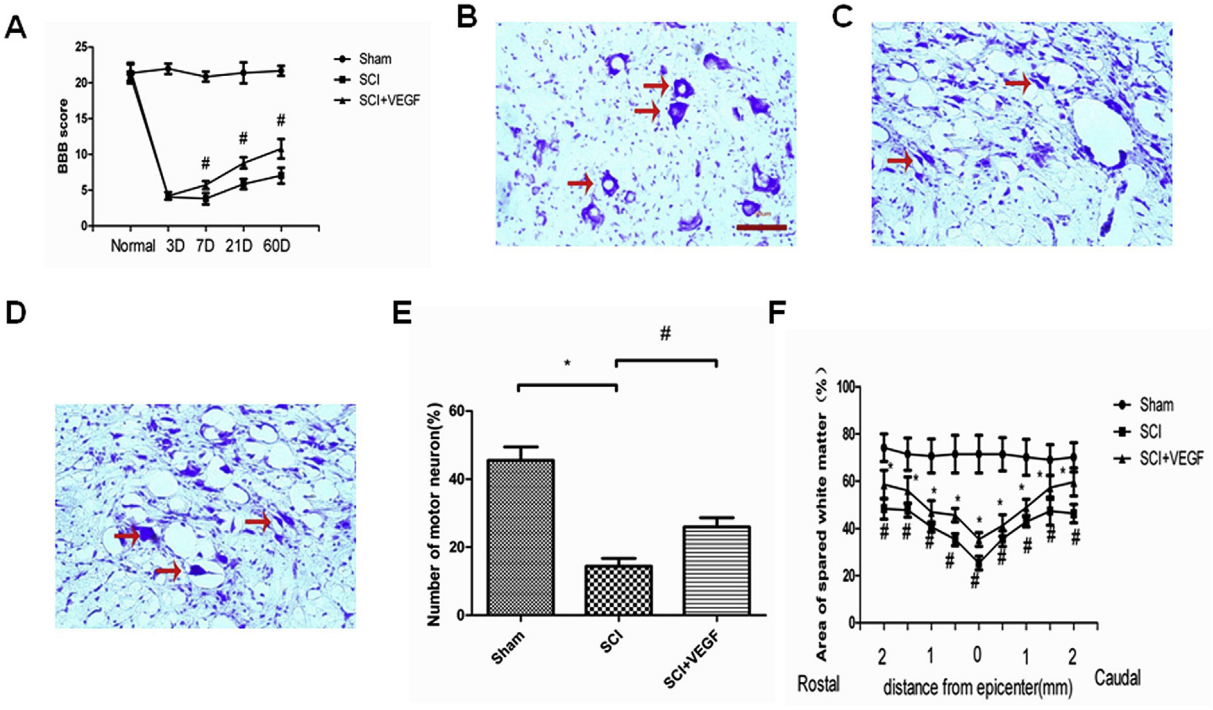

Fig. 1. VEGF promotes functional neurobehavioral recovery after SCI (A)VEGF treatment group obtained a significantly higher BBB score in comparison to SCI group ($p < 0.05$) at 7 days, 21days and 60days. #$p < 0.05$ SCI + VEGF versus the SCI group. Nissl staining of motor neuron in large anterior horn in the Sham group(B), SCI group(C) and SCI + VEGF group(D). (E)Number of motor neuron cell accounts in the anterior horns (F) Percentage of area of spared white matter up to 2 mm rostral and caudal to the injury epicenter Values are means ± SD * $P < 0.05$ SCI versus the normal control group. #$p < 0.05$ SCI + VEGF versus the SCI group.

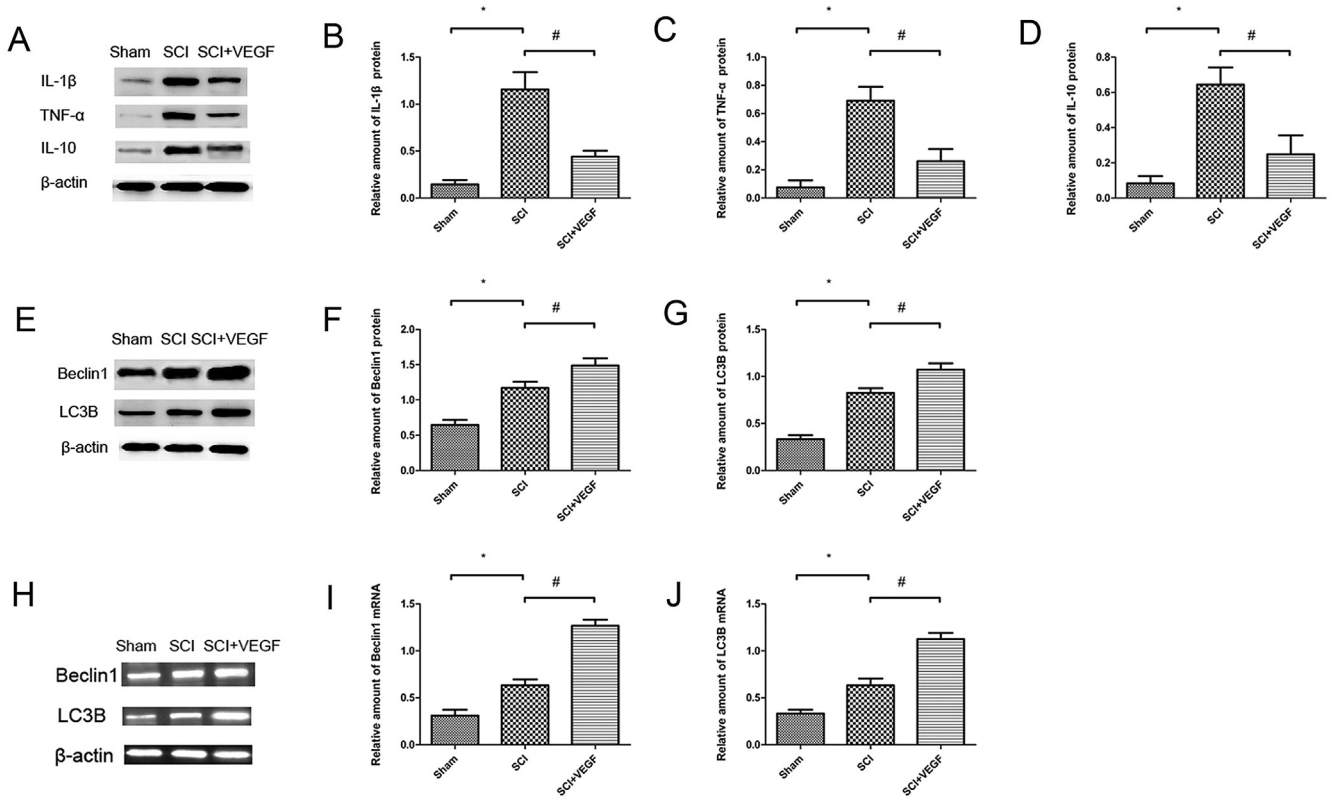

Fig. 2. VEGF administration decreased the expression of inflammation factors IL-1β, TNF-α and IL-10, up-regulated autophagy related protein Beclin1 and LC3B. (A)Western blot of IL-1β, TNF-α, IL-10 and β-actin protein expression at 7D after SCI and VEGF administration (BCD) Quantitative analysis of the levels of IL-1β, TNF-α and IL-10 expression. (EH) Western blot and RT-PCR results of Beclin1 and LC3B expressions at 7D after SCI and VEGF administration. (FGIJ) Quantitative analysis of protein and mRNA of Beclin1 and LC3B expression. The band densities were normalized to β-actin. Values are means ± SD * $P < 0.05$ SCI versus the normal control group. #$p < 0.05$ SCI + VEGF versus the SCI group.

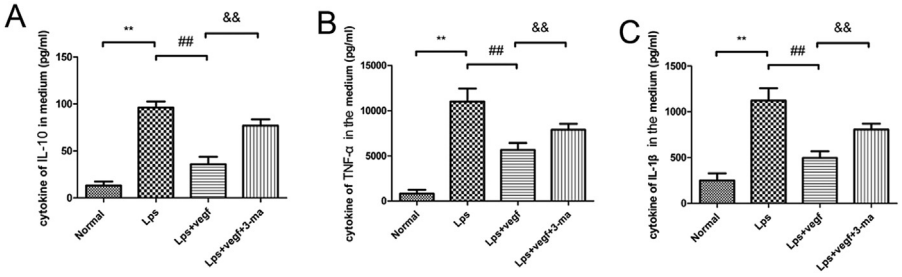

Fig. 3. Cytokine of IL-10, TNF-α, IL-1β in medium of neuron-glia co-cultured response to lipopolysaccharide (LPS) exposing. ELISA of IL-10, TNF-α, IL-1β and β-actin protein expression at 1D after LPS and VEGF administration. (ABC) Quantitative analysis of the levels of IL-10, TNF-α and IL-1β expression. The levels of IL-10, TNF-α and IL-1β were significantly increased by LPS. VEGF significantly reduced the level of IL-10, TNF-α and IL-1β. However, it become increased again when autophagy was inhibited by 3-MA. **$P < 0.01$ LPS versus the normal control group. ##$p < 0.01$Lps + vegf versus the lps group. && $p < 0.01$ Lps + vegf+3-ma group versus the Lps + vegf group.

angiogenesis and improves neurobehavioural outcomes [17–19]. The aim of this work was to elucidate the protective role of VEGF in SCI by examining the inflammation response and autophagy changes. A better understanding of the effects of VEGF on autophagy in SCI will provide a new perspective to treat SCI. Our study showed a single micro-infusion of $VEGF_{165}$ reduced inflammation response and increased the expressions of Beclin1 and LC3B at early stage in SCI. VEGF treatment also reduced inflammation response which was partially reversed by 3-MA. VEGF increased the autophagy related proteins in neuron-glia co-cultured cells treated by LPS. Taken together, these data suggested that the neuronal protective role of VEGF may be at least partly attributed to the activation of autophagy and the decrease in the inflammation response.

Immediately after injury, neutrophils are recruited from the circulation and CNS glia (astrocytes and microglia) are activated within the first 24 h after SCI [20,21]. The release of pro-inflammatory cytokines attract inflammatory cells move toward the injury site to remove damaged tissues [22,23]. Expression of the pro-inflammatory markers IL-6, IL-1β, TNF-α and IL-12 increases acutely in response to SCI [24,25], the inflammatory response leads to a release of vasoactive mediators, such as IL-1β, leading to BSCB disruption [26]. Evidence suggests that the endothelium transport systems may be modified due to SCI demonstrated by the upregulation of the specific transporter for TNF-α [27]. Our results show that IL-1β, TNF-a and IL-10 were up-regulated after SCI, however, VEGF significantly decreased the expressions of IL-1β, TNF-α and IL-10. In vitro study, cytokines in the medium of co-cultured cells

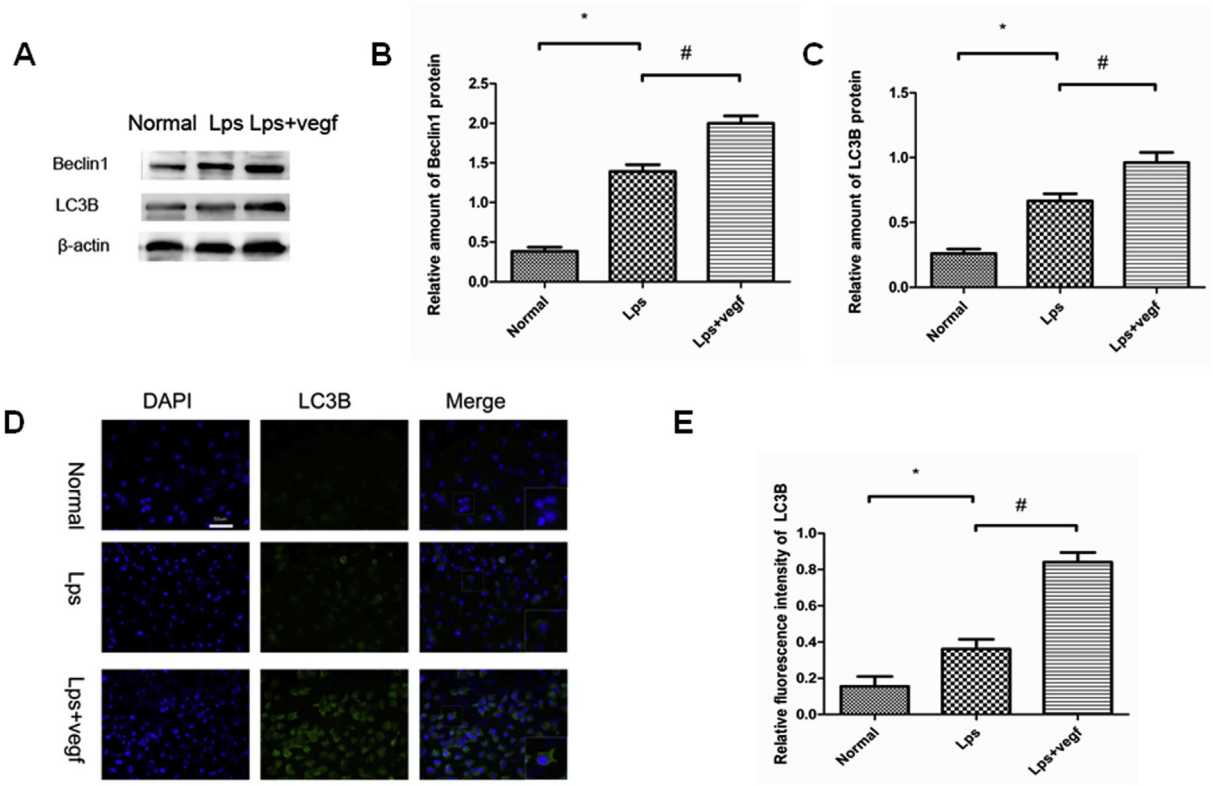

Fig. 4. VEGF administration increased proteins of Beclin1 and LC3B in neuron-glia co-cultured cells in LPS induced toxicity. (A) Western blot of Beclin1 and LC3B and β-actin protein expression at 1D after LPS and VEGF treatment. (BC) Quantitative analysis of the levels of Beclin1 and LC3B expression. The band densities were normalized to β-actin. Values are means ± SD (D) Immunofluorescence Staining of LC3B at 1D after VEGF pretreatment in neuron-glia co-cultured cells in LPS induced toxicity. (arrows show the positive cells, scale bar = 50 μm) Results of LC3B (green) and nuclei are labeled with DAPI and neurons with obvious LC3B signals are identified using red arrowheads. (E) Quantitative analysis of relative fluorescence intensity of LC3B.*P < 0.05 LPS versus the Normal control group. #p < 0.05 Lps + vegf versus the Lps group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly increased treated by LPS, pretreated with VEGF significantly decreased the level of IL-1β, TNF-α and IL-10. However, it was reversed when treated by 3-MA, the autophagy inhibitor (Figs. 2 and 3).

The disruption of vascular injures in SCI leads to tissue ischemia and edema, causing neuronal death and break off the tracts [28]. VEGF promotes the condensation of endothelial cells (ECs) into blood vessel termed vasculogenesis [29,30] and promotes neutrite extension in spinal cord. VEGF enlarged neuron cell bodies and extended dentrites by increasing MAP2 expression,the marker of neurons [31]. After SCI, VEGF was upregulated in astrocytes and immune cells [32]. However, consistent down-regulation of VEGF was found in the injured tissues during the first four weeks after injury [33,34]. VEGFR-1 and VEGFR-2 were found expressed in neurons and astrocytes, and subsequent up-regulation in microglia/macrophages and reactive astrocytes were discovered at the injured site [35]. We use a 24 h delayed intervention strategy after SCI because studies using acute applications exhibit unfavorable effects of VEGF exacerbates secondary damage of SCI [36]. Our data shows that VEGF treatment significantly improved BBB score of SCI rats and decreased motor neuron loss and improved the spared areas of white matter (Fig. 1).

Autophagy has been shown to be increased after SCI. The lipidated active form of LC3 (LC3B) becomes inserted into the inner and outer membranes of the phagophore and the autophagosome until its fusion with the lysosome. LC3 regulates autophagosome development and is used extensively as an autophagosome marker. Beclin1 plays important role in promoting autophagy through binding to a bcl-2-interacting protein. Autophagy may serve as a protection against apoptosis in mechanically-injured spinal cord neurons through mTOR signaling or enhancing Beclin-1 expression [37]. Autophagy is critical for limiting cell growth and promoting cell survival in times of stress [38]. Autophagy promotes survival of EPCs by inhibiting apoptosis in hypoxia and promotes proliferation and differentiation of EPCs which is critical for angiogenesis of the ischemic tissue [39]. Autophagy induction and inhibition was correlated with VEGF-mediated angiogenesis [40]. Although the primary role of autophagy is to protect cells, it paradoxically can also have a role in cell death [41]. Our work confirms the notion that VEGF treatment activated autophagy related protein Beclin1 and LC3B and decreased the inflammation response after the injury. In vitro study, VEGF increased expression of autophagy related protein Beclin1 and LC3B and decreased the inflammation factors IL-1β, TNF-α and IL-10 (Fig. 4).

The present study showed that VEGF administration increases the autophagic flux and attenuates inflammation response in the *in vivo* and *in vitro* studies. This finding may reveal a new molecular basis for VEGF neuroprotective roles, and may provide a novel approach to treat SCI.

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Transparency document

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References

[1] C.A. Oyinbo, Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade, Acta Neurobiol. Exp. (Wars) 71 (2011) 281–299.
[2] A.R. Blight, Miracles and molecules–progress in spinal cord repair, Nat. Neurosci. (5 Suppl) (2002) 1051–1054.
[3] U. Graumann, M.F. Ritz, O. Hausmann, Necessity for re-vascularization after spinal cord injury and the search for potential therapeutic options, Curr. Neurovasc Res. 8 (2011) 334–341.
[4] N. Ferrara, H.P. Gerber, J. LeCouter, The biology of VEGF and its receptors, Nat. Med. 9 (2003) 669–676.
[5] K. Jin, Y. Zhu, Y. Sun, X.O. Mao, L. Xie, D.A. Greenberg, Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11946–11950.
[6] L. Erskine, S. Reijntjes, T. Pratt, L. Denti, Q. Schwarz, J.M. Vieira, B. Alakakone, D. Shewan, C. Ruhrberg, VEGF signaling through neuropilin 1 guides commissural axon crossing at the optic chiasm, Neuron 70 (2011) 951–965.
[7] C. Ruiz de Almodovar, P.J. Fabre, E. Knevels, C. Coulon, I. Segura, P.C. Haddick, L. Aerts, N. Delattin, G. Strasser, W.J. Oh, C. Lange, S. Vinckier, J. Haigh, C. Fouquet, C. Gu, K. Alitalo, V. Castellani, M. Tessier-Lavigne, A. Chedotal, F. Charron, P. Carmeliet, VEGF mediates commissural axon chemo attraction through its receptor Flk1, Neuron 70 (2011) 966–978.
[8] M. Sondell, G. Lundborg, M. Kanje, Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system, J. Neurosci. 19 (1999) 5731–5740.
[9] T. Licht, R. Eavri, I. Goshen, Y. Shlomai, A. Mizrahi, E. Keshet, VEGF is required for dendritogenesis of newly born olfactory bulb interneurons, Development 137 (2010) 261–271.
[10] T. Licht, I. Goshen, A. Avital, T. Kreisel, S. Zubedat, R. Eavri, M. Segal, R. Yirmiya, E. Keshet, Reversible modulations of neuronal plasticity by VEGF, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 5081–5086.
[11] B. Li, W. Xu, C. Luo, D. Gozal, R. Liu, VEGF-induced activation of the PI3-K/Akt pathway reduces mutant SOD1-mediated motor neuron cell death, Brain Res. Mol. Brain Res. 111 (2003) 155–164.
[12] L. Van Den Bosch, E. Storkebaum, V. Vleminckx, L. Moons, L. Vanopdenbosch, W. Scheveneels, P. Carmeliet, W. Robberecht, Effects of vascular endothelial growth factor (VEGF) on motor neuron degeneration, Neurobiol. Dis. 17 (2004) 21–28.
[13] L. Tolosa, M. Mir, V.J. Asensio, G. Olmos, J. Llado, Vascular endothelial growth factor protects spinal cord motoneurons against glutamate-induced excitotoxicity via phosphatidylinositol 3-kinase, J. Neurochem. 105 (2008) 1080–1090.
[14] Y.R.L.B. Tovar, R. Tapia, VEGF protects spinal motor neurons against chronic excitotoxic degeneration in vivo by activation of PI3-K pathway and inhibition of p38MAPK, J. Neurochem. 115 (2010) 1090–1101.
[15] M. Martinez-Vicente, A.M. Cuervo, Autophagy and neurodegeneration: when the cleaning crew goes on strike, Lancet Neurol. 6 (2007) 352–361.
[16] M. Kundu, C.B. Thompson, Autophagy: basic principles and relevance to disease, Annu Rev. Pathol. 3 (2008) 427–455.
[17] Y. Liu, S. Figley, S.K. Spratt, G. Lee, D. Ando, R. Surosky, M.G. Fehlings, An engineered transcription factor which activates VEGF-A enhances recovery after spinal cord injury, Neurobiol. Dis. 37 (2010) 384–393.
[18] C.B. Patel, D.M. Cohen, P. Ahobila-Vajjula, L.M. Sundberg, T. Chacko, P.A. Narayana, Effect of VEGF treatment on the blood-spinal cord barrier permeability in experimental spinal cord injury: dynamic contrast-enhanced magnetic resonance imaging, J. Neurotrauma 26 (2009) 1005–1016.
[19] J. Widenfalk, A. Lipson, M. Jubran, C. Hofstetter, T. Ebendal, Y. Cao, L. Olson, Vascular endothelial growth factor improves functional outcome and decreases secondary degeneration in experimental spinal cord contusion injury, Neuroscience 120 (2003) 951–960.
[20] D.J. Donnelly, P.G. Popovich, Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury, Exp. Neurol. 209 (2008) 378–388.
[21] K.D. Beck, H.X. Nguyen, M.D. Galvan, D.L. Salazar, T.M. Woodruff, A.J. Anderson, Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment, Brain 133 (2010) 433–447.
[22] M.L. Novak, T.J. Koh, Phenotypic transitions of macrophages orchestrate tissue repair, Am. J. Pathol. 183 (2013) 1352–1363.
[23] J.E. Burda, M.V. Sofroniew, Reactive gliosis and the multicellular response to CNS damage and disease, Neuron 81 (2014) 229–248.
[24] G. Nocentini, S. Cuzzocrea, T. Genovese, R. Bianchini, E. Mazzon, S. Ronchetti, E. Esposito, D.P. Rosanna, P. Bramanti, C. Riccardi, Glucocorticoid-induced tumor necrosis factor receptor-related (GITR)-Fc fusion protein inhibits GITR triggering and protects from the inflammatory response after spinal cord injury, Mol. Pharmacol. 73 (2008) 1610–1621.
[25] A. Sato, H. Ohtaki, T. Tsumuraya, D. Song, K. Ohara, M. Asano, Y. Iwakura, T. Atsumi, S. Shioda, Interleukin-1 participates in the classical and alternative activation of microglia/macrophages after spinal cord injury, J. Neuroinflammation 9 (2012) 65.
[26] O.N. Hausmann, Post-traumatic inflammation following spinal cord injury, Spinal Cord. 41 (2003) 369–378.
[27] W. Pan, A.J. Kastin, Increase in TNFalpha transport after SCI is specific for time, region, and type of lesion, Exp. Neurol. 170 (2001) 357–363.
[28] D.N. Loy, C.H. Crawford, J.B. Darnall, D.A. Burke, S.M. Onifer, S.R. Whittemore, Temporal progression of angiogenesis and basal lamina deposition after contusive spinal cord injury in the adult rat, J. Comp. Neurol. 445 (2002) 308–324.
[29] P. Carmeliet, V. Ferreira, G. Breier, S. Pollefeyt, L. Kieckens, M. Gertsenstein, M. Fahrig, A. Vandenhoeck, K. Harpal, C. Eberhardt, C. Declercq, J. Pawling, L. Moons, D. Collen, W. Risau, A. Nagy, Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele, Nature 380 (1996) 435–439.
[30] N. Ferrara, K. Carver-Moore, H. Chen, M. Dowd, L. Lu, K.S. O'Shea, L. Powell-Braxton, K.J. Hillan, M.W. Moore, Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene, Nature 380 (1996) 439–442.
[31] J.M. Rosenstein, J.M. Krum, C. Ruhrberg, VEGF in the nervous system, Organogenesis 6 (2010) 107–114.
[32] D. Bartholdi, B.P. Rubin, M.E. Schwab, VEGF mRNA induction correlates with changes in the vascular architecture upon spinal cord damage in the rat, Eur. J. Neurosci. 9 (1997) 2549–2560.
[33] M.F. Ritz, U. Graumann, B. Gutierrez, O. Hausmann, Traumatic spinal cord injury alters angiogenic factors and TGF-beta1 that may affect vascular recovery, Curr. Neurovasc Res. 7 (2010) 301–310.
[34] J.J. Herrera, O. Nesic, P.A. Narayana, Reduced vascular endothelial growth factor expression in contusive spinal cord injury, J. Neurotrauma 26 (2009) 995–1003.
[35] J.S. Choi, H.Y. Kim, J.H. Cha, J.Y. Choi, S.I. Park, C.H. Jeong, S.S. Jeun, M.Y. Lee, Upregulation of vascular endothelial growth factor receptors Flt-1 and Flk-1 following acute spinal cord contusion in rats, J. Histochem Cytochem 55 (2007) 821–830.
[36] R.L. Benton, S.R. Whittemore, VEGF165 therapy exacerbates secondary damage following spinal cord injury, Neurochem. Res. 28 (2003) 1693–1703.
[37] Z.Y. Wang, J.H. Lin, A. Muharram, W.G. Liu, Beclin-1-mediated autophagy protects spinal cord neurons against mechanical injury-induced apoptosis, Apoptosis 19 (2014) 933–945.
[38] R.C. Wang, B. Levine, Autophagy in cellular growth control, FEBS Lett. 584 (2010) 1417–1426.
[39] H.J. Wang, D. Zhang, Y.Z. Tan, T. Li, Autophagy in endothelial progenitor cells is cytoprotective in hypoxic conditions, Am. J. Physiol. Cell. Physiol. 304 (2013) C617–C626.
[40] J. Du, R.J. Teng, T. Guan, A. Eis, S. Kaul, G.G. Konduri, Y. Shi, Role of autophagy in angiogenesis in aortic endothelial cells, Am. J. Physiol. Cell. Physiol. 302 (2012) C383–C391.
[41] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature 451 (2008) 1069–1075.