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HIF-1α/VEGF signaling pathway may play a dual role in secondary pathogenesis of cervical myelopathy

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ABSTRACT

Cervical spondylotic myelopathy (CSM) is one of the most common spinal cord disorders affecting the elderly. Yet the exact pathophysiology of CSM remains unclear. Vascular response to initial mechanical compression and associated ischemia may involve in secondary pathophysiology. Chronic compressive lesions to cervical cord resulting in lack of perfusion have established considerable evidences to support ischemia as an important pathogenesis both in patients and animal models, a similarity as that of acute spinal cord injury (SCI). In hypoxic condition following SCI, the up-regulation of vascular endothelial growth factor (VEGF), is consistent with increasing hypoxia induced factor-1α (HIF-1α) in acute periods. HIF-1α/VEGF signaling pathway is thought to play a dual role following SCI. In one hand, VEGF was demonstrated to be correlated with angiogenesis (protecting vascular endothelial cells, increasing blood vessel density and improving regional blood flow), neurogenesis (antiapoptotic, neurotrophic, attenuate axonal degradation), and locomotor ability improvement. In other hand, some studies revealed that VEGF have limited therapeutic effect, even exacerbate the secondary damage following SCI. VEGF administrations in acute or subacute periods result in elevation of blood-spinal cord barrier (BSCB) permeability even last for chronic course. BSCB permeability elevation initiates a secondary cascade of events involving excitotoxicity, infiltration of leukocytes and tissue edema. With comprehensive understanding of temporal and spatial of HIF-1α/VEGF signaling pathway, development of therapeutic strategies to promote new vessel growth while minimize the deleterious effects of VEGF-induced microvascular permeability, and thereby improve neurologic function, seems to be feasible and promising.

Introduction

Cervical spondylotic myelopathy is one of the most common spinal cord disorders affecting the elderly. Yet the uncertainty of CSM pathophysiology, i.e. insidious onset, variety of clinical signs, symptoms and the lack of pathognomonic findings, poses a big challenge for clinicians to diagnose and refer to surgery [1]. The simple pathoanatomic concept that a narrowed spinal canal causes compression of the enclosed cord, leading to local tissue ischemia, injury, and neurological impairment, fails to explain the entire spectrum of clinical findings observed in CSM patients. Recently, a new chronic spinal cord compression model in rat was developed to mimic clinical scenario for studying pathophysiology of CSM [2]. We implanted a water-absorbing polymer in canal of cervical spine of rat, which expanded slowly in vivo to induce chronic compressive injury of spinal cord [2]. In rats with severe neurological deficit, cell number in gray matter and myelin in white matter was significant less with more blood vessels in comparison with those with moderate neurological deficit. The preliminary findings suggested that vascular response to initial mechanical compression and associated ischemia may involved in secondary pathophysiology. The exact mechanism of vascular events in natural course of spinal cord chronic compressive injuries remains to be elucidated.

Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor specifically activated by hypoxia [3]. The accumulation of HIF-1α in ischemic or hypoxic tissues might promote adaptive mechanisms for cell survival and was found to be an important mediator of hypoxia-induced tolerance to ischemia, which then mediates a series of transcriptional responses [4]. VEGF is the downstream target gene of hypoxia inducible factor-1α (HIF-1α), which was reported to promote angiogenesis by inducing migration and proliferation of endothelial cells primary. As a potential stimulator of angiogenesis, VEGF can improve locomotor function in hypoxia condition following SCI [5,6]. In the contrary, hypoxia inducible VEGF can increase vascular permeability or disrupt BSCB [7,8]. However, most of these discoveries were base on acute SCI. little

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is known about the role of HIF-1α/VEGF pathway in secondary pathophysiology of chronic spinal cord compression lesion.

Hypothesis

HIF-1α/VEGF signaling pathway plays a dual effect in cervical myelopathy, i.e. promotes angiogenesis, neurogenesis, and neuroprotective effect, while elevates BSCB permeability and thereby initiates a secondary cascade of events involving excitotoxicity and infiltration of inflammatory cytokines.

Evaluation of the hypothesis

HIF-1α/VEGF signaling pathway

As a target gene of HIF-1α, VEGF transcription in hypoxic cells is up-regulated by hypoxia induced factor-1α (HIF-1α), which binds to a 28 base pairs element in the 5′ promoter region, the hypoxia response element (HRE) [9]. Within the HRE, an HIF binding segment and a downstream HIF ancillary sequence are required for VEGF transcriptional activation [10]. VEGF primary binds two related receptor tyrosine kinases (RTKs) include VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1). Systemic hypoxia or transient ischemia results in induction of HIF-1α and VEGF in neuronal and glial, and upregulation of VEGFR-1 and VEGFR-2 as well as their downstream signaling molecules PI3K and Akt [11]. Expression of VEGFR-1 (Flt-1) through paracrine release signaling is upregulated by hypoxia via HIF-1 dependent mechanism and binds neuropilins, so as to play a novel role in neuroprotection or neurotrophic [11]. VEGFR-2 is the major mediator of mitogenic, angiogenic and permeability enhancing effects of VEGF.

HIF-1α-mediated up-regulation of VEGF occurs under hypoxic conditions. Localization and hypoxic induction of VEGF was examined in the spinal cord of transgenic mice carrying a mutation in the superoxide dismutase-1 gene [12]. In rat spinal cord, the number of cells expressing HIF-1α and VEGF increased rapidly from 16 to 20 weeks after radiation injury, before white matter necrosis and forelimb paralysis [13]. HIF-1α expression in the radiation-induced injury revealed a key cellular response to hypoxia and the presence of a key inducer of hypoxia-responsive genes [13]. Recently, VEGF receptors are demonstrated to upregulated in microglia/macrophages and reactive astrocytes in the vicinity of the lesion in the contused spinal cord and almost all reactive astrocytes expressing VEGF-1 or VEGF-2. It suggests that VEGF may be involved in inflammation and in the astroglial reaction via specific VEGF receptors following acute spinal cord contusion in rats [14].

HIF-1α/VEGF disrupts BSCB integrity and deteriorates neurologic function

The blood-spinal cord barrier (BSCB) is located at the level of the capillary, and is composed of specialized endothelial cells surrounded by basal lamina. It regulates the fluid microenvironment of the spinal cord, thus play an important role in the pathophysiology of SCI [15]. Molecules transportation is regulated and restricted based on the integrity of BSCB. Disruption of BSCB exposes the spinal cord to a cytokines and vasoactive substances, leading to vasogenic edema and neurologic impairment in SCI [15].

VEGF is not only potent and specific for vascular endothelial cell proliferation but also important for vascular permeability [7,8]. VEGF expression in experiment SCI was examined usually in the acute phase or even last for subacute phase [14,16]. These findings implicated that increment of BSCB permeability is correlative to VEGF expression in the acute or subacute phase of SCI. Therefore, it seems predictable that exogenous VEGF administration in the acute or subacute phase of SCI will increase permeability of BSCB in theory. In recent years, various therapeutic attempts of exogenous VEGF administrations explore its effect. These attempts included deliver methods (intraparenchymal, intrathecal and intravenous), deliver temporal (acute or subacute phase, but not chronic phase), and dose.

A microinjection into gray matter with 2 μg exogenous recombinant human VEGF-165 (rhVEGF165) showed that the permeability of BSCB increased in 30 min and maximized at 72 h. The elevation of BSCB was found to associate with histopathological exacerbation and tissues loss [7]. It suggested that intraparenchymal application of the proangiogenic factor VEGF may exacerbate SCI, likely through its effect on vessel permeability. Similarly, Patel et al. [8] placed a gelfoam contain 4 μg VEGF on the lesion site immediately after SCI and found that the permeability of BSCB was significantly increase up to day 56. In contrast, Herrera et al. [17] conducted an injection of adeno-associated virus mediated VEGF-165 combined with angiopoietin-1 into the lesion epicenter of contusion spinal cord immediately after SCI in rats. Partial restoration of BSCB integrity near the lesion epicenter was observed after 28 and 56 days, and locomotor improvement in day 56. It seemed to reveal that this therapeutic strategy maintains BSCB integrity in the chronic course of SCI and correlates with functional recovery. However, the BSCB architecture repairman is probably contributed to angiogenesis with vascular stabilization, the effect of angiopoietin-1 rather than VEGF-165 administration. Exogenous VEGF administration on the lesion site appears to be significant increase BSCB permeability [9]. Disruption of BSCB leads to further vasogenic edema, disruption of oxygen transport and hypoxia, and even a vicious cycle including catecholamines, excitotoxic amino acids, arachidonic acid release, free-radical production, and lipid peroxidation. However, in chronic spinal cord compression, one study using semi-quantitative RT-PCR to detect the expression of VEGF mRNAs showed that there was not significant difference between the control and compression groups [18].

HIF-1α/VEGF improves spinal cord angiogenesis and neurogenesis

Increased levels of VEGF were detected around spinal injury sites [19]. VEGF-treated animals had an increased amount of spared tissue in the lesion center and a higher blood vessel density in parts of the wound area compared with controls and reduced functional impairment [5]. Ex vivo VEGF delivery by neural stem cells also increased the density of blood vessels and enhanced tissue sparing in the injured spinal cord. These pathological changes were correlated with improved locomotor. The multifaceted effects of VEGF on endogenous gliogenesis, angiogenesis, and tissue sparing could be utilized to improve functional outcomes following SCI [6]. In a more recent investigation of a clip compression of rat spinal cord model, elevated levels of VEGF were maintained for at least 6 weeks by intraparenchymal injection of a viral vector containing a transcription factor which induces VEGF expression [20]. Vascularization in chronic spinal cord compressive lesion, a reactive hypervascularity with thickening of the walls of the intermedullary arteries and capillaries occurs in response to chronic vascular insufficiency [21], may also be similar to that in SCI. VEGF may play an important role in angiogenesis, regulation of blood flow, and protection of endothelial cells in response to ischemia after SCI.

Recently, VEGF has emerged as a novel role of neurotrophic factor. After spinal cord injury, activation of VEGF and its receptors increase blood vascular density, restore blood supply, promote neuronal survival, as well as axonal regeneration and functional recovery. Numbers of studies indicated that protective effects observed with VEGF delivery were attributable to protection/repair of blood vessels, decreased apoptosis and possibly also by other additional effects on glial cells or certain neuron populations [5].

Indeed, higher blood vessel densities and increased axonal regeneration were presented in spinal cord injury simultaneously and correlatively. VEGF was demonstrated to be a potent neurotrophic factor which has a protective effect on the injured neurons [22]. Treatment of the spinal cord parenchyma with anti-VEGF antibody decreased the number of surviving neurons around the epicenter of a contusion injury, indicating a neuroprotective role of endogenous VEGF [19]. This treatment resulted in enhanced tissue sparing within a range of a millimeter distance from the lesion epicenter and a mild improvement of motor function [20], as measured by the BBB locomotor scale. However, van Neerven et al. [23] concluded that repetitive intrathecal VEGF delivery has limited therapeutic effects on spinal cord injury, thermal hypersensitivity of the hindpaws and motor deficits remained unaffected. Interestingly, low dose (0.5 μg) and high dose (20 μg) VEGF delivery were reported to have no significant improvement in locomotor ability [5]. These neuroprotective effects are mediated by activation of the intracellular tyrosine kinase domains. It influences several downstream signaling pathways, including mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt [24–26]. Binding of VEGF-A to VEGFR-2 receptor on neurons stimulates dimerization of VEGFR-2 and neuropilin-1, which activate PI3-K/Akt and extracellular regulated ERK-1/-2 signaling pathways. The PI3-K/Akt pathway is an important regulator of cell proliferation and survival. It has been shown to mediate the anti-apoptotic effects of VEGF in endothelial cells [26], as well as to transducer neuroprotecive effects in an immortalized neuronal cell line.

Conclusions

The pathophysiology change including ischemia, excitotoxicity, apoptosis and demyelination were all seen in chronic spinal cord compressive lesion. HIF-1α/VEGF signaling pathway plays complex rules under ischemia and hypoxia condition in SCI. However, the role of hypoxia-induced HIF-1α/VEGF pathway in blood-spinal cord barrier following chronic spinal cord compressive lesion remains unclear. Further research is required to elucidate the mechanisms underlying hypoxia-induced VEGF in BSCB disruption in CSM. With an increased understanding of the pathophysiological mechanisms involved in CSM, strategies that can be developed to block BSCB disruption and improve neurologic function.

Conflict of interest statement

None declared.

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