Role of vascular endothelial growth factor and other growth factors in post-stroke recovery

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Abstract

Stroke is a major health problem world-wide and its burden has been rising in last few decades. Until now tissue plasminogen activator is only approved treatment for stroke. Angiogenesis plays a vital role for striatal neurogenesis after stroke. Administration of various growth factors in an early post ischemic phase, stimulate both angiogenesis and neurogenesis and lead to improved functional recovery after stroke. However vascular endothelial growth factors (VEGF) is the most potent angiogenic factor for neurovascularization and neurogenesis in ischemic injury can be modulated in different ways and thus can be used as therapy in stroke. In response to the ischemic injury VEGF is released by endothelial cells through natural mechanism and leads to angiogenesis and vascularization. This release can also be up regulated by exogenous administration of Mesenchymal stem cells, by various physical therapy regimes and electroacupuncture, which further potentiate the efficacy of VEGF as therapy in post stroke recovery. Recent published literature was searched using PubMed and Google for the article reporting on methods of up regulation of VEGF and therapeutic potential of growth factors in stroke.

Key Words

Angiogenesis, ischemic stroke, vascular endothelial growth factors

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Ann Indian Acad Neurol 2014;17:1-6

Introduction

Stroke is a global health problem and is the second most common cause of death and a leading cause of disability world-wide.[1,2] Ischemic stroke generates a hypoxic condition in the brain thus leading to dysfunctioning of brain tissue in that area. Restoration of local blood flow by angiogenesis can reverse the ischemic environment and lead to long-term recovery.[3]

Angiogenesis is the key feature of neuronal post stroke reorganization and stroke recovery. Folkman[4] introduced the concept of angiogenesis as a necessity of tumor growth. Brain ischemia itself induces angiogenesis through hypoxia inducible factor 1 (HIF-1),[5] a transcription factor that respond to the changing intracellular O_2 concentration and induces erythropoietin (EPO) expression. Angiogenesis is activated through release of polypeptide growth factors and cytokines and specific up-regulation of the angiogenic factors involves transforming growth factor-beta (TGF-β), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)-2 in response to ischemic stroke, but VEGF is the most potent hypoxia inducible angiogenic factor amongst all[6] which is secreted by endothelial cells and pericytes. VEGF itself is up-regulated by other growth factors within hours of stroke and has a strong influence on growth of new blood vessels in the injured area of the brain.[7] Its production constitutes adaptive response to hypoxia, which promotes angiogenesis in post stroke events and eventually leads to functional recovery.[8]

This article will discuss the role of growth factors with special reference to VEGF in post-stroke, the different ways to modulate VEGF and interpret/predict their efficacy as future therapy module in attaining post stroke recovery.

Access this article online	
Quick Response Code:	Website: www.annalsofian.org
	DOI: 10.4103/0972-2327.128519

Role of Growth Factors in Angiogenesis

bFGF

bFGF plays an important role in angiogenesis *in vivo*. It mediates endothelial cell migration, proliferation and differentiation into tube-like structures. bFGF protects against hypoxic-ischemic insult *in vitro* and *in vivo*.[9]

bFGF may promote angiogenesis both by a direct effect on endothelial cells and also indirectly by the up regulation

of VEGF in vascular smooth muscle cells.[10] It stimulates protease production in cultured capillary endothelial cells. It also stimulates deoxyribonucleic acid synthesis and motility in capillary endothelial cells and induces angiogenesis *in vivo*. The acidosis caused by hypoxic and ischemic conditions enhances VEGF and bFGF messenger ribonucleic acid (mRNA) expression as well as bFGF secretion.[11] Thus, bFGF and VEGF have a synergistic effect on angiogenesis *in vivo*. This has been shown by combined administration of VEGF and bFGF which stimulated greater and more rapid augmentation of collateral circulation, resulting in superior hemodynamic improvement compared with either VEGF or bFGF alone.[12]

Tumor Necrosis Factor-alpha: (TNF-alpha)

Ischemic and other insults can induce increases in TNF-alpha levels in the human brain. Acute increases (1-6 h) in TNF-alpha mRNA and protein expression are observed after experimental brain injury in rat.[13]

Direct evidence for neuroprotective effect of TNF-alpha comes from TNF receptor (TNFR1 or TNFR2) knockout mice. Enhanced injury was observed in TNFR1 deficient but not in TNFR2 deficient mice after ischemia of brain, thereby suggesting that TNFR1 receptor signal transduction confers a neuro-protective effect.[14] TNF-alpha exerts its neuro-protective action via activation of nuclear factor-κB.

Timing of TNF-alpha production also influences its neuro-protective effects. The prolonged presence of unbound TNF-alpha also induces pathologic cellular changes in a receptor-independent fashion. mRNA levels of VEGF, bFGF, interleukin (IL-8) and their receptors increased after human micro vascular endothelial cells were exposed to TNF-alpha. Thus, TNF-alpha induced angiogenesis appears to be modulated through angiogenic factors, such as VEGF.[15]

Angiopoietin

The angiopoietin/tie receptor system may contribute to angiogenesis and vascular remodeling by mediating interactions of endothelial cells with smooth muscle cells and pericytes as cerebral ischemia results in the induction of both angiopoietin-1 and angiopoietin-2 genes. However, the temporal profiles of their expression are different.[16]

Angiopoietin-2 has been shown to work in concert with VEGF at the front of invading vascular sprouts by blocking the action of constitutively expressed angiopoietin-1, allowing vessels to remain in a more plastic state in response to sprouting signal provided by VEGF. Hence, angiopoietin-2 is associated with vessel sprouting and angiopoietin-1 stabilizes the vasculature during angiogenesis.[17]

VEGF Signaling

VEGF was first described as a vascular factor by Senger *et al*.[18] and then recognized as angiogenic factor by Leung *et al*.[19] VEGF consists of gene family that includes seven members, Placental growth factors, VEGF A, VEGF B, VEGF C, VEGF D, VEGF E and VEGF F, each member contains a signal sequence that cleaves during its biosynthesis. By different splicing, 4 different isoforms of the VEGF exists *in vivo,* VEGF 206, VEGF 189, VEGF 165 and VEGF 121.[20]

VEGF family members ligand have three receptor protein kinases VEGF R1 (flt1), VEGF R2 (kinase insert domain receptor-Flk-1) and VEGF R3 and two non-enzymatic receptors (neutrophilin-1 and neutrophilin-2).[21]

The expression of VEGF R2 and VEGF R1 is effected by hypoxia, although to a lesser extent than that of VEGF. Transcription of VEGF R1, but not that of VEGF R2 is enhanced by hypoxia through a post-transcriptional mechanism.[22]

The SHP-1 and SHP-2 protein tyrosine phosphatases physically associate with VEGF R2 after stimulation with VEGF, thus participating in the generation and modulation of VEGF-induced signals.[23]

Heparin binding form of VEGF can bind to the cell surface and extracellular matrix (ECM)-associated heparin-sulfate proteoglycan and can release angiogenic factors such as bFGF which are stored in heparin-sulphates of ECM. This observation is significant because VEGF and bFGF synergies with respect to their ability to induce angiogenesis.[24]

Eventually activation of VEGF receptors results in generation of proteases (e.g. collagenase, plasminogen activators [PA] and PA inhibitor-1) that are required for the breakdown of blood vessels that are required for the breakdown of basement membrane in the first step of angiogenesis,[25,26] in the expression of specific integrins required for angiogenesis and finally in the initiation of cell proliferation and cell migration. VEGF also activates focal adhesion kinase and associated proteins that have been shown to maintain survival signals in endothelial cells.[27]

Nitric oxide (NO) also up regulates VEGF expression. It contributes to the blood-vessel permeability effects of VEGF and to VEGF-mediated vasodilation. A transient augmentation and redistribution of cerebral blood flow were observed in the ischemic lesion after early and late administration of VEGF respectively, suggesting that exogenous VEGF generates NO in ischemic brain.[28]

A study also suggests the role of transcription factors signal transducers and activators of transcription (STAT)-1 and STAT-3 in modulating VEGF expression in the vascular smooth muscle cells. STAT-1 suppresses HIF-1 alpha expression whereas STAT-3 positively regulates HIF-1 alpha expression and thus down regulates and up regulates the VEGF expression respectively.[29]

Perlecan is a heparin sulfate proteoglycan in the brain matrix degraded by various proteolytic and glycolytic enzymes. Heparin sulfate is removed from perlecan by heparin sulfatase and protein core is digested by stromelysin and collagenases into smaller fragments,[30] cysteine proteases Cathepsin Land Cathepsin B activates perlecan from full length perlecan[31] and generates its fragment having domain V at the time of ischemia which interacts with alpha (5) beta (1) integrin in the brain endothelial cells, leading to increased phosphorylation of ERK, which leads

to the subsequent activation and stabilization of eIF4E and HIF 1 alpha, thus promoting brain angiogenesis by inducing VEGF release from brain endothelial cells following stroke.[32]

Production of VEGF by Endogenous Factors

VEGF-A and VEGF R2 receptor perform a central role in angiogenesis, neurogenesis and neuroprotection by increasing delivery of both oxygen and energy substrate and thus participates in brains endogenous response to ischemic injury.[33] Other members of family like VEGF B, is also known to be induced by experimental stroke and limits ischemic brain injury.[34]

In the ischemic brain, the macrophages, neurons and glial cells appear to contain VEGF. Macrophages in the periphery and in core of early stage of infarct become the first main source of VEGF. Macrophages also participate in angiogenesis; a macrophages derived peptide PR39, inhibited the ubiquitin-proteosome dependent degradation of HIF-1 alpha protein, resulting in accelerated formation of vascular structure *in vitro*.[35] Ischemic neurons have also been found to contain VEGF. These neurons could secrete VEGF under hypoxic conditions along with endothelial cells.[36]

Many cytokines and growth factors have been shown to modulate VEGF gene expression. IL-6 produced locally by resident brain cells plays an essential role in post stroke angiogenesis. Increased expression of these genes leads to increased angiogenesis and improved cerebral blood flow during delayed phase of the stroke, thus conferring improved long term outcome with reduced lesion size. IL-6 preconditioning of neural stem cells was found to induce secretion of VEGF from these stem cells through activation of signal transducer and activation of transcription.[37] Platelets also contribute to tumor induced angiogenesis as platelets are the carrier of angiogenic growth factors including VEGF.[38]

Certain indirect angiogenic cytokines, such as TGF-β 1, may act via induction of bFGFs and VEGF gene expression in the cells resident near endothelial cells *in vivo*. Hypoxia constitutes a potent stimulus for VEGF gene expression but does not regulate bFGF under the same experimental conditions.[39]

EPO plays an important role in angiogenesis through up regulation of VEGF/VEGF receptor system, both directly by enhancing neovascularization and indirectly by recruiting endothelial progenitor cells (EPCs). It also significantly increases brain derived neurotrophic factor (BDNF) in ischemic area.[40] Endogenous prostaglandin E2 also up regulates VEGF expression by activation of EP4 receptors and heals indomethacin-induced small intestinal lesions.[41] Androgens such as dihydrotestosterone and testosterones acting on androgen receptor stimulate cell proliferation in primary human aortic endothelial cells through up regulation of VEGF in time and dose dependent manner.[42]

Exogenous Administration of VEGF and Its Role in Post-ischemic Stroke Recovery

Hypoxia itself induces an increase of VEGF expression in ischemic area of brain but this endogenous VEGF secretion is inadequate to entirely protect the brain injury. VEGF plays pivotal role in angiogenesis *in vivo* thus therapeutic cerebral angiogenesis to enhance collateral vessel formation in ischemic area using VEGF, which is a specific mitogen for endothelial cells can be a potential method for cerebral revascularization. Intraventicular injection of VEGF antibody found to increases the infarct volume after focal cerebral ischemia in rats, suggesting that expression of neural VEGF may be one of the neuroprotective mechanisms.[43]

VEGF when administered not only diffused into and accumulated in adjacent brain parenchyma but remained intact for some time[44] and produced significant cerebral angiogenesis and immunoexpression of flt-1 (VEGF R1) receptors. *In vivo* neuroprotection of ischemic brain by exogenous VEGF does not necessarily occur with angiogenesis; instead neuroprotection may be greatly compromised by doses of VEGF capable of inducing angiogenesis. Thus VEGF enhances vascular proliferation in dose dependent manner.[45]

In animal model when VEGF is applied topically it unmasks the protective action of VEGF by avoiding its deleterious effects on vascular permeability. Topical application of VEGF to the cortical surface as well as intramuscular injection of VEGF reduces infarct volume and brain edema after temporary middle cerebral artery occlusion (MCAO)[46] and this effect is mainly due to the neuroprotective function VEGF in cerebral ischemia. Not only in adult but also in neonatal rats VEGF given 5 min after reoxygenation following hypoxic ischemia reduces brain injury but in neonatal rats VEGF has small therapeutic window unlike in adult rats.[47] Pre-morbid status of the patient is also an essential criteria for their selection for VEGF therapy,[48] e.g., if stroke patients may suffer from pre-existing chronic diseases such as diabetes or hypertension which can complicate therapeutic angiogenesis because these diseases directly affect blood vessels of nervous tissues. Determination of the optimal dose of VEGF, route of administration, time of administration and its combination with other growth factors will provide more effective way in post-stroke recovery.

Combined Role of Stem Cells and Growth Factors Post Stroke

Mesenchymal stem cells (MSCs) improves functional deficit after stroke as bone marrow derived mesenchymal stem cells (BMSCs) secretes distinctively different cytokines and chemokines such as VEGF, Insulin growth factor-1, endothelial growth factor, angipoietin-1, EPO etc., which are known to enhance wound healing in ischemic area.[49]

It is studied that transplantation of the VEGF gene modified MSCs may provide more potent autologous cell transplantation therapy for stroke than transplantation of BMSCs alone. When telomerized MSC are transfected with BDNF, Glial derived growth factors (GDNF) and ciliary neurotrophic growth factor genes using fiber-mutant adenovirus vectors it leads to significant functional recovery and reduces ischemic damage with more efficacy than treatment with MSCs alone and effect can be seen even when it is applied 6 h after infarction. This method also maintains exceptionally high level of neurotrophic growth factors, e.g., BDNF during critical post ischemic period

which contributes to enhanced neuroprotection.[50] Thus growth factors and stem cells work synergistically in functional restoration and angiogenesis post-stroke.[51]

Vascular Epo/EpoR system also plays an important role in ischemia-induced angiogenesis in mice *in vivo*. This system induces post-ischemic angiogenesis, secretion of VEGF from ischemic muscle and BM-derived cells, enhances VEGFR-2 expression in ischemic tissue and recruits BM-derived pro-angiogenic cells to ischemic tissue.[42]

Survival and regenerative capabilities of transplanted BMSCs can be enhanced by hypoxic preconditioning of the BMSCs. Hypoxic conditions induce angiogenesis in post-ischemic brain is vital for successful stem cell transplantation as it provides nutrient and oxygen to the cell so that cells can survive and become functional. Hypoxic exposure to the cells also up regulates HIF-1 alpha, VEGF, BDNF and GDGF and their receptors.[52] BMSCs treatment have extended therapeutic window which creates an opportunity to treat most if not all stroke patients as BMSCs transplanted 1 month after stroke also increases brain plasticity and improve long term functional outcome. Thus, this therapeutic approach may be used beyond hyper acute phase of stroke.[53]

Transplanted stem cells may secrete human vascular endothelial growth factor which induce neurovascularisation in spatio-temporal manner in peri-infarct region at 2 weeks post transplantation and influence tissue already undergoing repair and revascularization and restore impaired Blood Brain Barrier (BBB) on its sub-acute delivery.

Intra-arterial transplantation of the vascular cells, i.e., embryonic cells and mural cells derived from the human embryonic stem cell in mice contributes to vascular regeneration and provide therapeutic benefit for ischemic brain after MCAO as transplanted cells modulates the production of major angiogenic factors like VEGF, bFGF and PDGF and their receptors thus promotes functional outcome post-stroke by reducing infarct area.[54]

Thus stem cells and their products play a pivotal role in partitioning off damage, safeguarding the tissue integrity and possibly promote regeneration in brain after stroke by growth factors up regulation but mechanism by which MSCs inhibits inflammation to facilitates its therapeutic effect is still to be resolved. Understanding the mechanism of cell therapy will assist in the improvement of therapeutic efficacy in stroke patients.

Exercise and VEGF

Exercise induces neurogenesis and angiogenesis through growth factors cascade. Functional capacities in the acute stroke patients have a major impact on the motor function, balance, mobility and activity of daily living. Regular exercise after stroke lead to functional recovery which sustains for long. Endurance exercise, i.e., running up regulates BDNF and synapsin I mRNA which helps to facilitate better outcome in patients with stroke.[55]

Regular exercise leads to augmentation of regional cerebral blood flow. Exercise preconditioning up regulates VEGF which further regulates expression of matrix metalloproteinase (MMP2) which degrades ECM. Further MMP2 facilitates conversion of pro-NGF and pro-BDNF in NGF and BDNF respectively in brain.[56] Altogether this pro-angiogenic factor leads to repair and restoration process of brain after ischemic event. Exercise also strengthens the micro vascular integrity after cerebral ischemia and up regulates endothelial NO synthesis, which improves endothelium function by up regulating VEGF expression.[57] Early exercise after MCAO improves blood flow capacity in the ischemic cortex and reduce infarct volume thus promote functional outcome.[58] In rat model it is found that physical exercise stimulates the uptake of other growth factors like insulin-like growth factor-1 and BDNF[59] which provide a simple mean to maintain brain function and promotes brain plasticity.

Thus exercise modulates endogenous angiogenic mechanisms and exerts its function in neurovascular remodeling mainly through VEGF which offers a potential breakthrough for development of new method for long-term recovery after stroke.

Electroaccupuncture

Various physiotherapy regimes are known to elevate the VEGF content and cerebral VEGF expression in rat model of stroke. EA treatment could promote neurovascularization after cerebral ischemia by up regulating VEGF which leads to mobilization, chemotaxis and homing of EPCs.[60] EA acts by up regulating the expression of angiogenic factors and down regulating the expression of antiangiogenic factors, thus EA is effective for post stroke functional recovery in rats by up regulating VEGF expression [Figure 1].[61]

Human Studies

In humans expression of VEGF was found to be significantly increased after acute ischemic stroke and recovery from stroke is associated with angiogenesis. VEGF reaches its peak 7 days

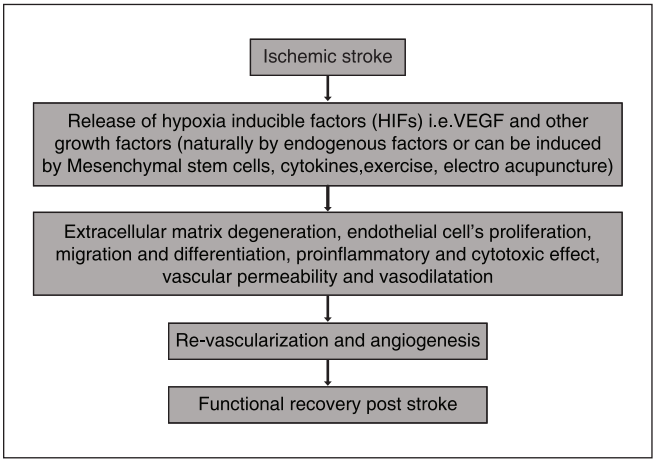

Figure 1: Schematic overview of post stroke release and action of growth factors

after stroke and remained elevated up to 14 days.[62] Mean VEGF expression was lowest in serum of patients with small infarct, increased in moderate infarct and was greatest in large infarct, which indicated that VEGF could be used as a marker of size of infarct.

The clinical significance of plasma VEGF values in neurological severity and functional outcome was different among stroke subtypes. Higher plasma values may be predictor of poor outcome in cardio embolic infarction and opposite trend was found in atherothorotic brain infarction patients (ATBI), thus significance of VEGF value in plasma in functional outcome may be different among different stroke subtypes.[63]

Serum VEGF level also correlated with long term prognosis in acute ischemic stroke patients, VEGF level increased in acute stage were found to be proportional to improved NIHSS score after 3 months. Thus VEGF can be used as biomarker in long-term prognosis of stroke as well.[64]

Conclusion

Use of growth factors to promote angiogenesis is emerging as a new therapeutic strategy for prevention and treatment of acute ischemic stroke as angiogenesis may restore surviving tissue longer and promote neural re-organization in affected area post-stroke. Various studies have been published on the different strategies for the treatment of stroke, but there is no ongoing clinical trial for stroke using VEGF therapy/VEGF combination therapy/angiogenesis therapy. VEGF may be used as stroke therapy as it leads to angiogenesis but angiogenesis has inverse relationship with neuroprotection, thus VEGF should be used in combinational therapy, where other neurotrophic factors and agents which are effective in reducing vascular permeability are included with the VEGF to reduce the adverse effects of neuroprotection. We have mentioned some of the various ways that helps in up regulation of the VEGF including exercise, physiotherapy regimes and combination of growth factors in stem cells post stroke etc. Further research studies need to emphasize on the Optimization of the minimal therapeutic dose, combination of growth factors to improve their efficacy, time of their administration and understanding the mechanism that how angiogenesis is regulated through number of pathways for identification of new therapeutic targets and modalities.

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How to cite this article: Talwar T, Srivastava MP. Role of vascular endothelial growth factor and other growth factors in post-stroke recovery. Ann Indian Acad Neurol 2014;17:1-6.
Received: 07-08-13, **Revised:** 02-10-13, **Accepted:** 01-12-13

Source of Support: Nil, **Conflict of Interest:** Nil