The Role of Angiogenesis in Damage and Recovery from Ischemic Stroke

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Opinion statement

Ischemic stroke is burdened with a high morbidity and mortality in our society. However, there are few effective and largely available therapies for this devastating disease. In additon to advancing acute reperfusion therapies, there is a need to develop treatments aimed to promote repair and regeneration of brain tissue damaged by ischemia (neurorecovery). Therapeutic angiogenesis and vasculogenesis represent novel approaches of regenerative medicine that may help in the cure of patients with acute ischemic stroke. Translation of our knowledge about these processes from the bench to bedside is still underway. Although angiogenesis (the sprouting of new blood vessels from pre-existing vascular structures) is likely to contribute to neurorepair, the finality of the angiogenic response in acute ischemic stroke has not been fully elucidated. The first therapeutic approach to angiogenesis after ischemic stroke would be the modulation of the endogenous angiogenic response. In this setting, early instauration of physical activity, statins, and peroxisome proliferator-activated receptor-γ agonists may enhance angiogenesis and neuroregeneration. Gene therapy with vascular growth factors has been successfully tested in patients affected by chronic myocardial and peripheral ischemia. Regarding brain ischemia, experiments in animal models have shown that the effect of these growth factors is critically affected by the dosage, route of delivery, and time of administration in relation to stroke onset. In addition, the optimal angiogenic substance is unknown. Finally, vectors for gene transfer should be further optimized. Therapeutic vasculogenesis consists of the administration of exogenous endothelial progenitor cells in order to enhance brain repair processes. Endothelial progenitor cells may be recruited in response to cerebral ischemia and participate in reparative vasculogenesis after acute ischemic stroke. Further research is needed to clarify their role and therapeutic applicability in human brain ischemia.

Introduction

Stroke is a leading cause of death and disability worldwide [1]. Ischemic stroke accounts for approximately 85% of all strokes. Despite its huge socioeconomic impact, there are still few therapeutic options available for this disease. The main objectives of acute ischemic stroke therapy are 1) to restore brain perfusion promptly enough to save ischemic tissue at risk of infarction (reperfusion therapies); 2) to preserve cerebral tissue viability and avoid hemorrhagic and medical complications of ischemic stroke (neuroprotection); and 3) to stimulate and induce

repair processes able to attenuate brain structural damage and to improve the functional outcome of patients with ischemic stroke (neurorecovery). Currently, only a small proportion of stroke patients benefit from acute reperfusion therapies. Moreover, most potentially neuroprotective drugs have proven to be ineffective in reducing brain infarct volume and related disability in stroke patients. Therefore, there is a need to investigate repair processes after cerebral ischemia in order to develop therapeutic strategies able to promote neurorecovery [2]. In this context, therapeutic angiogenesis and vasculogenesis hold promise to improve the prognosis of patients with ischemic stroke, although most of our knowledge in this field derives from experimental and animal models of cerebral ischemia.

Angiogenesis is a complex and finely regulated process triggered by hypoxia that consists of the sprouting of new blood vessels from pre-existing vascular structures. Animal and human studies have demonstrated that endogenous angiogenesis may play an important role in acute ischemic stroke improving brain tissue recovery and functional outcome [3–5]. These observations support the consideration of angiogenesis as a potential therapeutic target in acute ischemic stroke. However, there is controversy regarding the finality of the angiogenic response following acute cerebral ischemia: considered a mechanism involved in brain tissue regeneration for some investigators, others postulate that angiogenesis basically enables the infiltration of necrotic tissue by macrophages destined to cleaning up tasks [6], although these two opposite hypotheses might well be complementary. In addition, in our own experience angiogenesis may also be relevant in the natural history of diseases that cause chronic cerebral hypoperfusion, such as intracranial large-artery atherosclerosis [7].

Vasculogenesis is a related but differentiated process that consists of de novo vessel formation by bone marrow–derived endothelial progenitor cells (EPCs) [8]. In response to stimuli such as trauma or tissue hypoxia, EPCs are mobilized from bone marrow to peripheral blood and may participate in endothelial cell repair-regeneration and in tissue neovascularization processes [9]. In this context, experimental and human studies have shown that neovascularization is present in the adult brain exposed to ischemia and that EPCs participate in cerebral neovascularization processes in the adult brain of mice after ischemia [10,11]. Finally, we have first observed that a higher increment in the number of circulating EPCs during the first week after stroke onset is associated with a better outcome in patients with ischemic stroke [12]. Taken together, these findings suggest that EPCs may mediate neurorepair processes after cerebral ischemia, and that exogenous supplementation of EPCs to stroke patients might constitute a novel and promising therapeutic strategy.

INDICATIONS FOR HOSPITALIZATION: ROLE OF STROKE UNITS

Following international guidelines, all patients with acute ischemic stroke should be admitted to stroke units during the first days after stroke onset [13]. Stroke units allow 1) urgent administration of approved and investigational acute-phase treatments; 2) continuous specialized monitoring of neurologic status; 3) adequate acute management of blood pressure, glycemia, oxygenation, and temperature—conditions known to dramatically influence the basic processes involved in the development of ischemic damage and in the response to stroke therapies; and 4) early instauration of neurorehabilitation [14]. Following the acute phase, patients should be transferred to neurorehabilitation units.

Treatment

Diet and lifestyle: importance of physical activity

- Animal models of cerebral ischemia have shown that physical activity protects against ischemic stroke by reducing final infarct volume and improving functional outcomes [15,16,17••].
- Several mechanisms have been proposed that explain this beneficial effect:
  - Physical activity upregulates endothelial nitric oxide synthase, thus improving endothelial function [15].
  - Physical exercise increases the production and recruitment capacity of EPCs. Continuous voluntary running is associated with a higher number of circulating EPCs in animals and humans, which may imply a more robust angiogenic capacity [16].
  - Physical training after ischemic stroke is associated with higher numbers of newly generated vessels and sustained augmentation of cerebral blood flow within the ischemic brain tissue. Therefore, physical activity improves long-term stroke outcome in part by enhancing the angiogenic response [17••].

- Practical implications include the following:
  - Regular physical activity should be encouraged in the setting of stroke prevention. Sedentary lifestyle may be associated with worse outcomes [18].
  - Active neurorehabilitation should be initiated promptly after stroke onset. Further research is needed to establish the most adequate regimens of physical activity in terms of favoring neurorepair processes.

Pharmacologic modulation of the endogenous angiogenic response

- The endogenous angiogenic response is the result of the balance between many different stimulant and inhibitor factors that interact in an orchestrated manner [19••]. The main proangiogenic molecules may be classified as the following:
  - Growth factors: Vascular endothelial growth factor (VEGF) family, fibroblast growth factor family, angiopoietins, hepatocyte growth factor, platelet-derived growth factor, insulin-like growth factor family, and nerve growth factor. Each of these factors has its own specific function and regulation.
  - Inflammatory molecules: Angiogenic cytokines comprise monocyte chemoattractant protein-1, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor-α. The role of matrix metalloproteinases (MMP-9) is especially relevant.
  - Transcription factors: Hypoxia-inducible transcription factor and early growth response-1.
  - Pleiotropic substances: Tissue kallikrein, peroxisome proliferator-activated receptor (PPAR) activators, thrombin, nitric oxide, and secreted frizzled-related proteins.
- In contrast, other molecules are responsible for vessel destabilization and regression in order to finish the angiogenic process, once perfusion matches metabolic demand. The main antiangiogenic molecules include the following:
  - Growth factors: Thrombospondins, angiostatin, endostatin, and VEGF-soluble receptors Elt1 and Tie 2.
  - Inflammatory molecules: Interleukin-10 and C-reactive protein.
- The first approach to therapeutic angiogenesis for acute ischemic stroke would be the pharmacologic modulation of this complex endogenous angiogenic response. Statins and thiazolidinediones may be especially relevant in this field due to their effects on the expression of several angiogenic molecules.

Statins

Mechanism of action

Besides their cholesterol-lowering properties, statins exert a number of pleiotropic vasculoprotective and proangiogenic actions including 1) upregulation of endothelial nitric oxide synthase; 2) improvement of endothelial function; 3) upregulation of EPCs; and 4) induction of the expression of angiogenic factors such as VEGF. Through these and other effects, statins improve functional outcome and induce brain plasticity when administered in animal models of focal cerebral ischemia [20–23].

Standard dosage

High-dose atorvastatin (80 mg/d) has recently been proven to be efficacious in stroke secondary prevention [24]. However, its safety and efficacy profile in the setting of acute ischemic stroke have not yet been

tested. The MISTIC (Markers of Inflammation after Simvastatin in Ischemic Cortical Stroke) study was a small pilot, randomized, placebo-controlled clinical trial evaluating the safety and efficacy of simvastatin (40 mg/d) in the acute phase of ischemic stroke. Administration of simvastatin within the first 6 hours after stroke onset was safe and was associated with a better short-term clinical outcome [25].

Main side effects Statin toxicity is mainly related to muscular and hepatic side effects.

Special points Sudden discontinuation of chronic statin treatment leads to a rebound effect with downregulation of nitric oxide production, the so-called "statin withdrawal syndrome," which may be associated with worse stroke outcomes. Therefore, statin treatment should always be maintained from the first day after stroke onset [20].

Stroke-related dysphagia may limit statin bioavailability in the acute phase. Therefore, parenteral administration should be enabled in the future.

There is not enough level of evidence to recommend routine use of statins as neuroprotectants in the acute phase of ischemic stroke. Future research should address this issue.

PPAR-γ agonists: glitazones

Mechanism of action Recent experimental findings suggest that glitazones could have cytoprotective effects in acute cerebral ischemia. Among other mechanisms, PPAR-γ agonists may improve endothelial function independently of insulin sensitizing. Treatment with different glitazones may upregulate both the number and the functional capacity of EPCs [26,27]. However, the potential proangiogenic effect of thiazolidinediones has not been evaluated in acute cerebral ischemia.

Standard dosage Not yet tested in patients with acute stroke.

Main side effects Thiazolidinediones are hampered by adverse effects related to increased weight gain, fluid overload, and congestive heart failure [28].

Emerging therapies: therapeutic angiogenesis

Modalities of therapeutic angiogenesis

- Therapeutic angiogenesis postulates the local delivery of vascular growth factors to ischemic tissues as a way to foster new vessel formation. Several modalities have been proposed [19••]:
  - Delivery of growth factors as recombinant proteins.
  - Gene therapy: Based on the transfer of DNA sequences to somatic cells for therapeutic purposes. The gene transfer can be performed using viral vectors or with new nonviral methods.
  - Transfer of a single factor versus combined approaches (association of growth factors with different biological activities).

Therapeutic angiogenesis in patients with myocardial and peripheral ischemia

- Following successful application in animal models, therapeutic angiogenesis has been tested in clinical trials with patients affected by chronic myocardial and peripheral ischemia, in which conventional treatments were ineffective or technically not amenable. Results of the first clinical trials, mainly using VEGF or fibroblast growth factor gene transfer, demonstrate that therapeutic angiogenesis is safe, attenuates clinical symptoms, and improves indices of cardiac function [29–33].

Therapeutic angiogenesis in animal models of cerebral ischemia

VEGF

VEGF induces endothelial cells to proliferate, migrate, and survive. Gene transfer with VEGF for acute cerebral ischemia has been extensively researched in animal models of focal brain ischemia [34–41]. These studies have shown that the effect of VEGF on ischemic brain depends critically on the time of administration in relation to the onset of cerebral ischemia and on the route of delivery. As a safety concern, VEGF is able to increase vascular permeability, a step necessary to allow the migration of new endothelial cells, but involved in the processes of edema formation and hemorrhagic transformation of ischemic stroke. Accordingly, whereas early VEGF administration may increase the risk for acute stroke complications, delayed VEGF supplementation may be safe and able to promote neurorepair. Moreover, VEGF may exert deleterious actions in the infarct core and beneficial effects within the ischemic penumbra. Regarding the route of delivery, whereas intra-arterial or intravenous administration may increase blood–brain barrier leakage, direct application of VEGF to cortical surface or intracerebroventricular delivery seems to be associated with a neuroprotective-associated response.

Hepatocyte growth factor

Overexpression of hepatocyte growth factor after gene transfer into the brain attenuated ischemic injury in a rat model of focal cerebral ischemia by promoting angiogenesis, without cerebral edema [42].

Translation of therapeutic angiogenesis into clinical practice: main concerns

- The optimal angiogenic substance (or combination of substances) is still to be found.
- Dose and route of administration of angiogenic growth factors seem to be critical for their efficacy and safety profile.
- The finality of the angiogenic response in the setting of acute ischemic stroke has not been fully clarified. During the hyperacute phase of ischemic stroke, angiogenic factors such as VEGF may contribute to increase the permeability of the blood–brain barrier, which enhances the risk for hemorrhagic complications. In contrast, during the subacute phase, the promotion of angiogenesis within the ischemic penumbra may be beneficial to avoid the expansion of the ischemic damage. Finally, in the chronic phase angiogenesis may lead to the formation of a new capillary network within the infarcted tissue, which might allow the migration of neural and bone marrow–derived stem cells, thus favoring neuronal plasticity and neurogenesis. Therefore, the best time of administration should be defined for each growth factor.
- There is a need for further optimization of the vectors for gene transfer. Adenoviruses seem to be the most effective vectors, although the activation of immune reactions can lead to side effects or may preclude efficacy of future administrations. Plasmid vectors have limited infectivity potential, leading to low-level expression of curative genes [19••].

Emerging therapies: therapeutic vasculogenesis

EPCs in brain ischemia

- Vasculogenesis, the process by which angioblasts differentiate into endothelial cells to form de novo blood vessels, occurs not only in the early embryogenesis, but also in the adult brain in response to ischemia [10]. It has been shown that bone marrow–derived stem cells can migrate into the brains of adult animals and differentiate into astrocytes, microglia, neurons, and endothelial cells [43]. Moreover, intravenous administration of human bone marrow stromal cells to rats enhances angiogenesis in the ischemic boundary zone after stroke [44]. EPCs are a subclass of bone marrow–derived stem cells able to differentiate into endothelial cells; they have been shown to participate in neovascularization processes in the adult brain of mice after ischemia [10]. Recent research has demonstrated that circulating EPCs increase in response to cerebral ischemia in patients after acute ischemic stroke, and that the magnitude of this increase is directly related to a better functional outcome [12]. However, whether circulating EPCs are able to incorporate into brain ischemic areas and to promote regenerative vasculogenesis in humans remains to be clarified.

Modalities of therapeutic vasculogenesis

- We now refer to the transplantation of exogenous EPCs. Preliminary evidence suggests that EPC administration may have therapeutic use for the treatment of myocardial and peripheral ischemic disease in humans, although it has never been tested in patients with acute ischemic stroke [45–47]. The main modalities of therapeutic vasculogenesis are based on the following factors:
  - Source of EPCs: Isolated from adult peripheral blood, cord blood, or bone marrow.
  - Local or systemic EPC delivery.
  - Modulation of the endogenous EPC response (processes of recruitment from the bone marrow, migration, and infiltration of target tissue). We have previously mentioned how physical activity, statins, and PPAR-γ agonists may upregulate EPCs. Chemokine supplementation may also promote EPC mobilization and migration [48].
  - Manipulation of EPCs prior to administration: 1) Enrichment procedures, such as culture expansion, and 2) genetic manipulation to enhance EPC function. These manipulation procedures may be needed in stroke patients because ageing and vascular risk factors are associated with a decrease in number and functional capacity of autologous EPCs [49,50].
- *Special points*: A substantial loss of functional EPCs in the first few hours after transplantation has been described in coronary patients. Further research is needed to understand the causes and therapeutic impact of this phenomenon.

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