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Angiogenesis, neurogenesis and brain recovery of function following injury

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Abstract

Traumatic brain injury and stroke are major causes of mortality and morbidity worldwide. Unfortunately, almost all phase-III neuroprotective clinical trials for stroke and traumatic brain injury have shown no benefits; this has raised concerns regarding neuroprotective strategy alone as a therapy for acute brain injuries. There is therefore a compelling need to develop treatments that promote the repair and regeneration of injured brain tissue and functional recovery. Recent findings suggest that strategies to enhance angiogenesis and neurogenesis for brain injuries may provide promising opportunities to improve clinical outcomes during brain functional recovery. This article reviews current data on angiogenesis and neurogenesis in the adult brain after stroke and traumatic brain injury. Select cell-based and pharmacological therapies that promote angiogenesis and neurogenesis designed to restore neurological function after brain injuries are described. These findings highlight the need for a better understanding of injury- and therapy-induced angiogenesis and neurogenesis in the adult and suggest that the manipulation of endogenous neural precursors and endothelial cells is a potential therapy for brain injury.

Keywords

Angiogenesis; functional recovery; neurogenesis; neuroprotection; neurorestoration; neurovascular unit; stroke; traumatic brain injury

Introduction

Brain injuries caused by stroke and trauma remain major health problems worldwide, and are the leading causes of serious long-term disability. Focal ischemic stroke begins with a thrombus or embolus that occludes a cerebral artery. Ischemia also plays an important role in pathogenesis of traumatic brain injury (TBI). Pathophysiological responses in brain after stroke and TBI are highly complex and involve multiple mechanisms including excitotoxicity, free radical damage, and inflammation, leading to neuronal injury and cell death [1]. Currently, neuroprotection is a main strategy for the treatment of acute stroke and TBI. There are select excellent reviews on neuroprotection for stroke [2,3] and TBI [4–6]. Thus far, a monotherapy for saving neurons has not revealed any clinically effective neuroprotectants. Only one Food

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and Drug Administration (FDA)-approved drug, recombinant tissue plasminogen activator (tPA), for the treatment of clinical ischemic stroke shows therapeutic effects and this treatment is limited by its narrow therapeutic time window and related risks of brain hemorrhage [7]. Recent preclinical studies have revealed that brain injury induces neurogenesis (the generation of new neurons) and angiogenesis (the growth of new blood vessels). To the best of our knowledge, clinical trials in TBI and stroke have targeted neuroprotection and none of them have been aimed specifically at angiogenesis and neurogenesis. Agents and manipulations that boost angiogenesis and/or neurogenesis promote functional recovery after brain injuries [8]. This suggests that the manipulation of endogenous neural precursors and endothelial cells is a potential therapy for brain injury.

Neurogenesis and angiogenesis

Mammalian adult neurogenesis occurs in the subgranular zone (SGZ) of the hippocampus, subventricular zone (SVZ), and olfactory bulb (OB) [9,10]. Newly generated neuronal cells originate from neural stem cells (NSCs) in the adult brain. NSCs are the self-renewing, multipotent cells that generate the neuronal and glial cells of the nervous system [11]. Granule neurons in the dentate gyrus (DG) of the hippocampus continuously die and the progenitors may proliferate to maintain a constant cell number in the DG [12]. Similarly, the newly proliferated cells from SVZ replenish the dead OB neurons. In addition, the resident neural progenitors could be induced to replace neurons lost due to acute insults [13–15]. Newly generated neurons in the DG of the hippocampus are capable of projecting axons to the CA3 region in normal [10,16] and injured adult rats [17].

The adult brain vascular system is stable under normal conditions and is activated in response to pathological conditions including injuries [18]. Adult vascular remodeling includes angiogenesis by mature endothelial cells (growth of capillaries from pre-existing vessels) and by endothelial progenitor cells (EPCs). EPCs are present in the bone marrow and peripheral blood and are mobilized to peripheral blood after TBI. The number of $CD34^+$ EPCs in peripheral blood increases at 24 h, peaks at 48 h and returns to normal at 168 h after TBI. These $CD34^+$ cells are detected as early as 24 h after TBI in the area surrounding injured brain and the vessel-lumen structure with $CD34^+$ endothelial-like cell lining being observed at 72 h after TBI [19]. Bone marrow-derived endothelial progenitor cells (EPCs) also promote endothelial repair and contribute to ischemia-induced neovascularization [20]. Pharmacological agents such as statins, angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor blockers, PPAR-γ agonists and erythropoietin increase the number, mobilization and functional activity of EPCs [21]. However, some papers show that EPCs hardly incorporate into newly developed blood vessels and suggest that the cells may support pre-existing endothelium-derived angiogenesis by secreting growth factors including bFGF and VEGF [22]. The induction of angiogenesis can be achieved by delivering angiogenic factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and angiopoietins (Ang) [23]. Mobilization or transplantation of EPCs into ischemic tissues may emerge as a promising approach in the therapy of diseases associated with blood vessel disorders including stroke and TBI.

Central nervous system (CNS) neurovascular units (NVUs) are multi-cellular complexes consisting of endothelial cells, pericytes, neurons, and glial cells as well as growth factors and extracellular matrix proteins that are in physical proximity to the endothelium [24,25]. NVUs are niches for neural stem/progenitor cells (NSPCs) in the adult brain. Within the NVUs, newly born, immature neurons closely associate with the remodeling vasculature. The generation of new vasculature facilitates highly coupled neurorestorative processes including neurogenesis and synaptogenesis, which in turn lead to improved functional recovery [26–28]. Several compounds with angiogenic activities currently tested in clinical trials include sildenafil,

atorvastatin, erythropoietin (EPO) and carbamylated EPO (CEPO) [26]. Neurogenesis and angiogenesis are causally linked through vascular production of stromal-derived factor 1 (SDF1) and Ang1. Furthermore, SDF1 and Ang1 promote post-stroke neuroblast migration and behavioral recovery [29]. The disruption of the neurovascular coordination was observed in a variety of brain diseases such as infection, stroke, and trauma [30]. We and others demonstrate that injured brain can be stimulated to promote angiogenesis and neurogenesis [31–39], which are coupled restorative processes that contribute to functional recovery from stroke and TBI. Magnetic resonance imaging (MRI) indices of these neurorestorative events are highly correlative with neurologic function and may be used in real-time monitoring of recovery from stroke [40,41]. Some of the agents including EPO offer both neuroprotective and neurorestorative benefits [39,42–44].

Neurogenesis and angiogenesis after TBI and stroke

In the normal adult brain, SVZ cells migrate along the rostral migratory stream (RMS) to the OB where they differentiate into interneurons. There are several excellent reviews on neurogenesis after TBI [14] and stroke [13,15,45,46]. After stroke, neuroblasts generated in the SVZ migrate to the ischemic boundary zone (IBZ) where angiogenesis occurs, and during migration neuroblasts are closely associated with cerebral vessels [29,47]. Neuroblasts actively interact with the microenvironment to reach the ischemic striatum individually or in chains [48]. After migration, SVZ-derived neuroblasts differentiate into mature neurons in the IBZ [49–52]. Following injury activated endothelial cells of cerebral vessels secrete SDF-1α to attract neuroblasts expressing CXCR4, a receptor for SDF-1α [53–55], and blockage of CXCR4 abrogates migration of neuroblasts to the IBZ [29,53,55]. Experimental results show that stroke also induces neurogenesis in aged animals, although basal neurogenesis is attenuated in these animals [56–58]. Pharmaceutical agents such as statins and sildenafil substantially enhance angiogenesis and neurogenesis and improve functional outcome during stroke recovery in aged animals [58,59].

Cortical injury changes the migration routes of cells born in the SVZ of the adult brain from the RMS to injured areas. Cellular proliferation is found in the SVZ, corpus callosum, around the cortex, and subcortical areas anatomically connected to, but not directly injured by the impact in a rat TBI model [60]. Following TBI, cells from the SVZ can differentiate into neurons and glia in injured areas [61–63]. Brain injuries also stimulate neurogenesis in the SGZ of the hippocampus [34,39,63–66]. TBI induces hippocampal cell proliferation and the majority of the injury-induced cell population that survives for an extended period of time differentiates into mature granule neurons. Some mature granule neurons extend axonal projections into the CA3 region by 2 weeks post TBI [17]. The functional integration of the injury-induced population into the existing hippocampal circuitry appears as early as 2 weeks when cognitive recovery is observed in TBI rats [34]. In addition, there is a persistent proliferation of neurons and glia in the SVZ following brain trauma that does not diminish during aging (4 months up to 1 year) [67].

Endothelial cells in the IBZ proliferate as early as 12–24 h following stroke, leading to peri-infarcted angiogenesis 3 days following the ischemic injury [26]. Active angiogenesis is observed 3–4 days following the ischemic insult in humans [26]. Formation of neovessels in the adult brain after stroke and TBI is not restricted to angiogenesis [39,68,69] but also involves vasculogenesis contributed by circulating EPCs from bone marrow [19,20].

Angiogenesis and neurogenesis may play an important role in mediating functional recovery after experimental stroke and TBI [8,28,34,36,39,40,70,71]. Nearly all the neurorestorative agents that improve functional outcome after stroke and TBI increase angiogenesis and neurogenesis [8,40,44,71]. Specific ablation of adult-born hippocampal neurons impairs spatial

and object recognition memory in adult rats [72]. These data imply a direct and causal relationship between neurogenesis and recovery of neurologic function after brain injury. Brain angiogenesis may provide the critical neurovascular niches for neuronal remodeling. Understanding how neurovascular signals and substrates make the transition from initial injury to angiogenic and neurogenic recovery will be important for developing new therapies for brain injuries [73]. Coupling of angiogenesis and neurogenesis has also been observed in the adult human [74]. Human neurosphere-forming SVZ cells can self-renew and are multipotential [75,76]. Human adult neural stem/progenitor cells derived from the SVZ during routine surgery are capable of generating both neurons and astrocytes in vitro and may provide a source for application in cell-based human transplantation paradigms [77].

Erythropoietin

Erythropoietin (EPO) stimulates the maturation, differentiation and survival of hematopoietic progenitor cells [78,79]. While EPO and its receptor (EPORs) are only weakly expressed in normal adult brain, expression of EPO and the EPORs is greatly increased in neurons, neuronal progenitor cells, glia and cerebrovascular endothelial cells in response to many different types of cell injury [80,81]. Intraperitoneal administration of EPO (5000 U/kg) crosses the blood–brain barrier (BBB) and protects against brain injury in rats [37,42]. EPO treatment reduces ischemic infarct and hemorrhage volume, decreases neuronal death, and improves survival rates in animal models of stroke [82,83]. EPO may direct cell fate away from gliogenesis toward neurogenesis in neonatal stroke [84]. EPO administration at a dose of 5000 U/kg starting day 1 for 14 days after TBI significantly increases DG neurogenesis, and promotes restoration of spatial memory after TBI [34]. Post-TBI treatment (6 h or 24 h) with EPO (5000 U/kg) or CEPO (50 μg/kg) significantly increases BDNF expression and improves spatial learning at 5 weeks after injury in rats [85]. Efficacy of EPO is independent of increased hematocrit [44] and a multiple-dose EPO treatment (5000 U/kg daily for 3 days starting at day 1 post injury) increases functional recovery more than single-dose EPO therapy (5000 U/kg at day 1 post injury) in TBI rats [86]. EPO treatment initiated as late as 6 h post-TBI provides neuroprotection (i.e., decrease lesion volume and cell loss) as well as enhances neurogenesis, and subsequently improves sensorimotor and spatial learning functions [39,42–44]. Thus, EPO provides neuroprotection [39,42,43,87,88] and neurorestoration via promotion of neurogenesis and angiogenesis [34,37,44].

Treatment with EPO also contributes to neurovascular remodeling, leading to improved neurobehavioral outcomes after ischemic brain injury [37,89]. EPO enhances VEGF secretion from neural progenitor cells and neural progenitor cells treated with EPO upregulate VEGFR2 expression in cerebral endothelial cells, thus promoting angiogenesis [90]. While the therapeutic benefits of the novel EPO derivatives continue to be characterized in preclinical studies, the experimental findings in support for the use of EPO in human brain diseases have already been translated to clinical studies in acute ischemic stroke, chronic schizophrenia, and chronic progressive multiple sclerosis [83]. In stroke patients, EPO treatment may reduce infarct volume and improve functional outcomes [91]. It should be noted that the high EPO doses used for treatment of stroke and TBI significantly increase hematocrit [39,86]. The concern is that increased hematocrit may pose potential adverse vascular effects seen in the critically ill patients treated with EPO [92]. Although EPO administration has proven safe in animal studies and adult human patients, safety and efficacy data in neonates and infants are incomplete and long-term multi-center patient evaluations are necessary [82]. Nonhematopoietic EPO analogues such as CEPO are as effective as hematopoietic EPO without potential side effects [85,93–96]. CEPO at a dose of 50 μg/kg has a therapeutic window of at least 3 h and effectively improves clinical rating scores and motor function in a small clot embolic stroke rabbit model [95,96]. Stroke- and TBI-related EPO or CEPO clinical trials can be found on www.clinicaltrials.gov [97]. A Phase-I safety study of CEPO to treat patients with

acute ischemic stroke (AIS) has been completed (NCT00756249). Further safety and pharmacokinetic studies of CEPO are ongoing (NCT00870844). A Phase II/III multicenter efficacy study of EPO in AIS has been completed (NCT00604630) [98]. This clinical trial enrolled 522 patients with acute ischemic stroke with 460 patients treated within 6 h of symptom onset, at 24 and 48 h, with EPO infused intravenously (40,000 IU each). Systemic thrombolysis with recombinant tissue plasminogen activator (rtPA) was used in 63% of the stroke patients [98]. This is a negative trial that raises safety concerns, particularly in patients receiving systemic thrombolysis, but restorative effects of EPO should be pursued. However, we note, that to date, the interaction between EPO and rtPA has not been investigated. Also, this clinical trial points to the importance of fully evaluating CEPO and other EPO analogs for both acute protection and chronic restoration of function. A Phase II EPO combined with Beta-human chorionic gonadotropin in AIS trial has been completed (NCT00362414). A Phase III trial studying the effects of EPO on cerebral vascular dysfunction and anemia in TBI is underway (NCT00313716). A Phase II safety trial of Darbepoetin Alfa (a long-acting form of EPO) treatment in patients with severe TBI is ongoing (NCT00375869), as well as a Phase II/III study on the early administration of EPO to patients sustaining TBI (NCT00260052). A Phase III trial of EPO in ICU patients with TBI is being planned (NCT00987454). We are waiting for the reports from these clinical trials.

Cell therapy

Neuronal tissue has limited repair capability after injury. Cell therapies using NSPCs are promising approaches for the treatment of brain injury [99,100]. There are several excellent reviews on NSPC cell therapy for TBI [8,99–104]. NSPC therapy may replace lost brain cells, promote endogenous neurogenesis and improve functional recovery [100]. NSPCs also stabilize vasculature during ischemia, suggesting therapeutic application of NSPCs to promote revascularization and repair after brain injury [105]. There is little evidence to assess the applicability of NSPCs to brain injury patients, and well designed clinical trials are necessary to evaluate safety, toxicity and efficacy as well as optimal cell type, route and time of delivery for NSPCs [103,106].

Although embryonic stem cells or fetal tissues are suitable sources for cell therapy, their clinical application is limited by ethical considerations and other specific problems including tumorigenicity, viability, and antigenic compatibility [107]. Recent landmark experiments have shown that transient overexpression of a small number of transcription factors can reprogram differentiated cells into induced pluripotent stem (iPS) cells that resemble embryonic stem cells [108]. These iPS cells avoid the ethical issue inherent in embryonic tissues or oocytes and have the potential to generate patient-specific cell types for cell replacement therapy. iPS cells may offer promising opportunities for the treatment of brain injury. Mesenchymal stem cells (MSCs), mesoderm-derived cells, have been isolated from bone marrow, adipose tissue, umbilical cord blood, placenta and pancreas. MSCs exert powerful immunomodulatory effects, which include inhibition of proliferation and function of T cells, B cells, and natural killer cells, thus reducing immune reactions and increasing tolerance of MSC recipients [109]. This immunosuppressive property makes them an important alternative source for allogeneic cell therapy since MSCs can be isolated from donors, expanded and cryopreserved [28,110,111]. In this review, we focus on adult bone marrow-derived MSCs, which are able to give rise to neuronal cells and many tissue-specific cell phenotypes [28,112,113]. When grafted into the lateral ventricles of neonatal mouse brain, MSCs migrate and differentiate into OB granule cells and periventricular astrocytes [114]. Our studies indicate that systematically infused rat MSCs migrate into injured rat brain and survive [115–118]. Some implanted MSCs express the markers for neurons and astrocytes. MSC treatment significantly improves neurological functional recovery after TBI [115,119,120].

MSCs may provide neuroprotection when intrathecally given early (1 million cells, 6 h) in rats after TBI [121]. Delayed (24 h or 1 week after injury) administration of MSCs in the range of 2–8 million cells also significantly improves functional outcome after TBI and stroke [27,31, 35,116,122–126]. MSC therapy at 1 month after stroke (3 million cells, i.v.) shows therapeutic benefits [127]. CXC-chemokine receptor-4 (CXCR4) is expressed in MSCs and the interaction of SDF-1/CXCR4 may contribute to the trafficking of transplanted MSCs into injured brain [127]. MSCs secrete various growth factors including BDNF, VEGF and FGF, thus amplifying their endogenous brain levels [122,128–130]. MSCs also induce intrinsic parenchymal cells to produce these growth factors [129]. These trophic factors enhance angiogenesis and vascular stabilization in the lesion boundary zone, where the majority of MSCs that survive in the brain are located [119,124]. MSCs increase VEGF expression and promote angiogenesis after stroke [122,131–134]. These growth factors also promote neurogenesis [130,135,136]. MSCs not only increase vascular density in the lesion boundary zone and hippocampus [126] but also enhance neurogenesis in the SGZ and SVZ [35] in rodents after TBI. In addition, MSCs induce expression of bone morphogenetic proteins BMP2 and BMP4 and connexin 43 in astrocytes in injured brain, promoting synaptogenesis [137]. In concert with enhancing angiogenesis, neurogenesis, and synaptogenesis, MSCs significantly decrease glial scar formation and promote glial–axonal remodeling in rats after stroke [138]. Thus, MSC therapeutic benefits are probably not attributable to the very few MSCs that differentiate into brain cells [116]. However, MSCs appear to work as neurotrophic generators to promote brain functional recovery via angiogenesis, neurogenesis, synaptogenesis, and axonal remodeling (Figure 1). With delayed treatment, MSCs alone does not reduce the lesion volume after TBI [117], while collagen scaffolds populated with MSCs do reduce the lesion volume, foster the migration of MSCs into the lesion boundary zone, and improve spatial learning and sensorimotor function compared with MSCs alone after TBI in rats [139].

MSCs transferred with ex vivo hepatocyte growth factor gene are more therapeutically efficient for treating stroke rats than MSCs alone [140]. Cellular delivery of placental growth factor gene-modified MSCs provides better neuroprotection and angiogenesis, and better improvement in functional recovery in cerebral ischemia than MSCs alone [141]. Intravenous administration of human MSCs transfected with the Ang1 and VEGF genes shows the greatest structural-functional recovery as compared to monotherapy groups after cerebral ischemia [142]. Strategies to maximizing angiogenesis may prove valuable for brain injury therapies. Thus, genetically engineered MSCs and NSPCs with overexpression of growth factors may be an improved source for cell therapy for stroke and TBI. An excellent review of genetically modified stem cells used in experimental models of stroke can be found in reference [143].

Although the first clinical trial of autologous MSC therapy in stroke showed promising results [144], the optimal approach (types of MSCs, cell dose, timing of treatment, route of cell delivery) has yet to be determined. The safety and feasibility of autologous MSC treatment of TBI patients have been recently assessed [145]. No toxicity related to the cell therapy was observed within the 6-month follow-up period. Neurologic function was significantly improved by 6 months after the MSC therapy [146]. Clinical trials of cell therapy by intravenous injection of MSCs after ischemic stroke are ongoing (NCT00535197, NCT00761982, NCT00473057) and more are being planned (NCT00875654, NCT00908856) [97]. The safety of autologous stem cell treatment in children with TBI (NCT00254722) [97] is being examined. The potential short-term and long-term toxicities of MSCs still need to be determined before use in the clinic [146].

Statins

Statins, cholesterol biosynthesis inhibitors used for lowering cholesterol, show neuroprotective and neurorestorative benefits in animal models of TBI and stroke [71,72,147–151]. Many of

the pleiotropic effects of statins are cholesterol independent, such as improvement of endothelial function, increased nitric oxide (NO) bioavailability, antioxidant properties, inhibition of inflammatory responses, immunomodulatory actions, upregulation of endothelial NO synthase (eNOS), and decrease of platelet activation [37,59,152]. Statins induce angiogenesis, neurogenesis, and synaptogenesis and enhance functional recovery after stroke in rats [71]. VEGF, VEGFR2 and BDNF likely contribute to these restorative processes [148]. Oral administration of atorvastatin at a dose of 1 mg/kg daily for 14 days starting at 1 day after TBI significantly reduces the neurological functional deficits, increases neuronal survival [33,149,154] and synaptogenesis in the boundary zone of the lesion and in the CA3 regions of the hippocampus, and induces angiogenesis in these regions [148] and increases neurogenesis in the DG [33,70]. When administered in combination with MSCs, atorvastatin increases MSC access and/or survival within the injured brain and enhances functional recovery compared with monotherapy [155]. Statins induce neuroglial differentiation of human MSCs [156]. These cholesterol-lowering agents might be used in conjunction with MSC transplantation for treating neurological disorders and injuries.

Simvastatin therapy elevates the expression of BDNF and VEGF; increases cell proliferation and differentiation in the DG; and enhances the recovery of spatial learning after TBI [70]. Protective mechanisms for lovastatin may be partly attributed to dampening inflammatory response after TBI [147,154]. Simvastatin treatment provides long-lasting (3-month) functional improvement after TBI in rats. Lovastatin is currently approved by the FDA for the treatment of acute ischemic stroke patients 3 days after ictus and the maximum tolerated dose is estimated to be 8 mg/kg/day [157]. Another clinical trial showed that simvastatin-treated stroke patients improve significantly by the third day when simvastatin is given at 3–12 h after symptom onset (with an initial dose of 40 mg/day for the 1 week followed by a dose of 20 mg/kg until day 90 day) to 60 patients with cortical strokes. However, a non-significant increase in mortality and greater proportion of infections in the simvastatin group are the main safety concerns [158]. Therefore, a larger clinical trial is needed to confirm the net benefit of the statin therapeutic approach. It has been demonstrated that rosuvastatin (hydrophilic), but not simvastatin (lipophilic), provides end-organ protection in stroke-prone rats [159]. The property of hydrophilicity/hydrophobicity may contribute to the different statin pharmacology, for lipophilic statins are more susceptible to metabolism [160]. Consideration of the differences among the statins provides a rational basis for their preclinical research and clinical practice. Given the wide use of statins, their favorable safety profile and positive clinical data in patients, rare serious adverse events, and the extensive preclinical data showing neuroprotection and neurorestoration, further clinical studies are warranted to determine the neuroprotective and neurorestorative properties of statins after stroke and TBI. The effect of rosuvastatin on TBI-induced cytokine change is being studied in a clinical trial (NCT00990028) [97], as are the neuroprotective effects of lovastatin therapy on ischemic stroke recovery (NCT00243880) [97].

Phosphodiesterase type 5 inhibitors

Guanosine 3′, 5′-cyclic monophosphate (cGMP) acts as a relaxant second messenger in the blood vessels. cGMP-specific phosphodiesterase type 5 (PDE5) inhibitors elevate intracellular cGMP levels, increase cerebral blood flow and improve functional recovery after stroke [161,162]. Administration of a PDE5 inhibitor sildenafil (at a dose of 10 mg/kg administered subcutaneously 24 h after stroke and daily for an additional 6 days) to rats with embolic stroke enhances angiogenesis and selectively increases the cerebral blood flow level in the ischemic boundary, and improves neurological functional recovery compared to saline-treated rats [163]. MRI measurements, confirmed by histology, show that sildenafil treatment simultaneously enhances angiogenesis and axonal remodeling [164]. Sildenafil evokes neurogenesis and reduces neurological deficits when given to rats 2 or 24 h after stroke

[165]. Treatment with sildenafil at a dose of 3 mg/kg daily for 7 consecutive days starting 7 days after focal ischemia significantly enhances neurogenesis and improves functional recovery in aged rats after focal cerebral ischemia [58]. Treatment of ischemic stroke with a long-acting PDE5 tadalafil improves functional recovery, which is associated with increases of brain cGMP levels and enhancement of angiogenesis and neurogenesis [166]. Our recent clinical safety study of sildenafil shortly after ischemic stroke onset shows that sildenafil (25 mg daily for 2 weeks) appears to be safe in patients with mild to moderately severe stroke [167].

Conclusions

Brain injury induces angiogenesis and neurogenesis. Strategies to enhance angiogenesis and neurogenesis improve brain functional recovery in experimental stroke and TBI. A better understanding of cross talk between neurogenesis and angiogenesis will further lead to novel therapeutic avenues for TBI and stroke. Cell therapeutic intervention involves the stimulation of endogenous NSPCs or the transplantation of adult-derived NSPCs. NSPCs have been isolated from human post-mortem tissues, providing an alternative source of tissues for allogeneic cellular therapy which needs donor-recipient matching. Autologous transplantation does not require a matching donor and immune-suppressive drugs; NSPCs would be isolated from an undamaged area of the CNS, expanded, and grafted back to restore brain function. However, harvesting NSPCs from patients is time-consuming, may delay the therapy, and involves invasive surgery that destroys healthy brain tissue, limiting its clinical application. The select cell-based and pharmacological therapies (ie, MSCs, EPO, CEPO, statins) in the review induce endogenous neurorestorative remodeling by increasing angiogenesis, neurogenesis, and synaptogenesis, subsequently improving neurological functional recovery after stroke and TBI (Figure 1). However, several aspects should be considered during the preclinical studies and clinical trials in stroke and TBI. Prior to translation of an agent or cell into clinical trial, preclinical evidence should be sufficiently strong, based on multiple experiments, preferably in several models, and including optimal administration routes, single doses versus multiple doses, bolus dose versus continuous infusion, and therapeutic windows. Extensive pharmacokinetic data on agents for treating injured brain should also be obtained, ensuring adequate brain tissue penetration through the BBB. Timing for manipulation of these factors is also critical for achieving effective outcome after injury. In addition, effective translation of agents into clinical trials may require multiple functional agents including EPO, CEPO, statins or combination therapy. These potential combinations include agents (eg, pharmaceuticals or cytokines) with cells (eg, MSCs, neural stem cells, iPS cells, and genetically modified cells) or with other approaches (physical or electric stimulation). Combination of neuroprotective and neurorestorative therapy may facilitate recovery of the injured brain.

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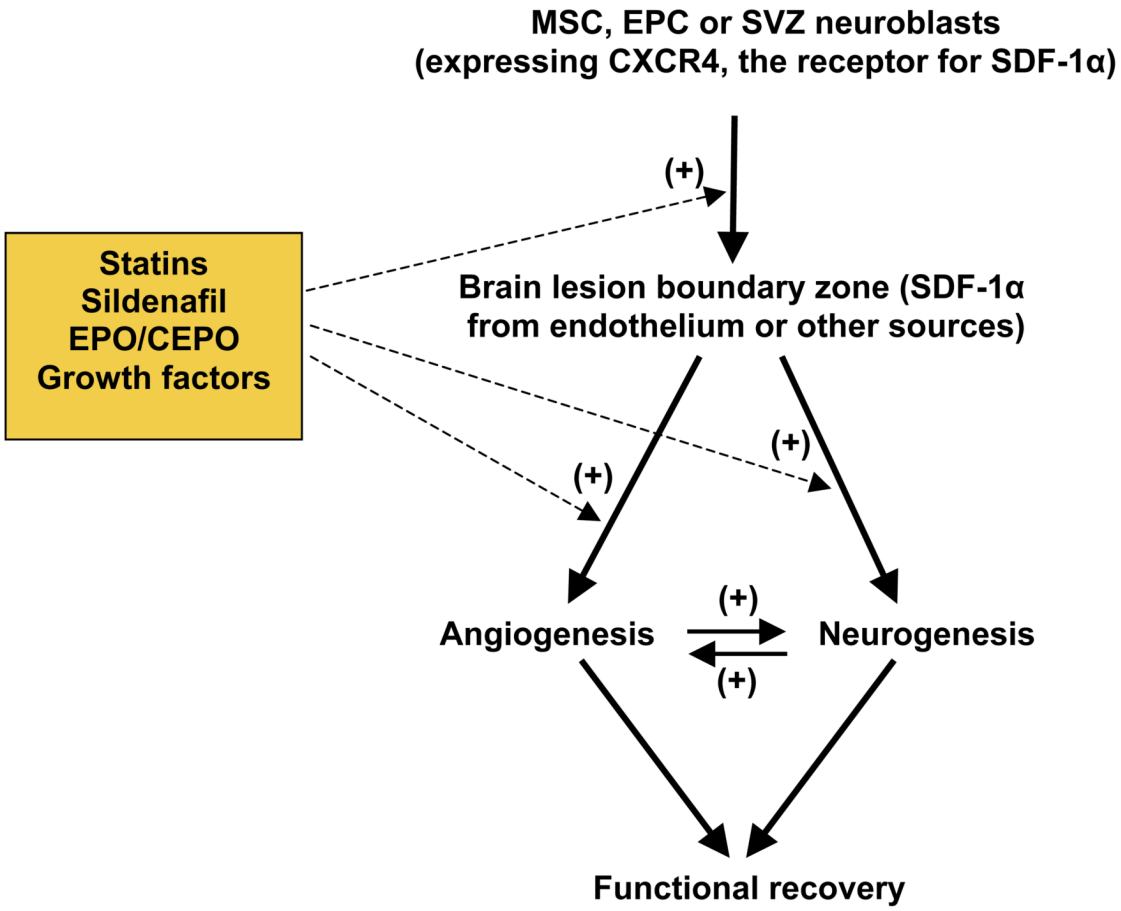

Figure 1. A simplified schematic diagram summarizing injury- and therapy-induced angiogenesis and neurogenesis after TBI and stroke

Following brain injury, CXC-chemokine receptor-4 (CXCR4)-expressing subventricular zone (SVZ) neuroblasts are attracted by increased stromal-derived factor 1 (SDF-1)α to migrate into the lesion boundary zone, where they differentiate into neural cells. Similarly, mesenchymal stem cells (MSCs) and endothelial precursor cells (EPCs) are directed to injured brain regions, where they secret growth factors to promote angiogenesis and neurogenesis. Erythropoietin (EPO) and statins promote the migration of SVZ neuroblasts into injured brain regions. Treatment with EPO/carbamylated erythropoietin (CEPO), statins, PDE5 inhibitors, MSCs, VEGF or basic FGF increases angiogenesis and neurogenesis after brain injury. In addition, angiogenesis and neurogenesis are coupled through VEGF, angiopoietins (Ang)1 and SDF1α. Brain remodeling, including angiogenesis and neurogenesis, may contribute to spontaneous and therapy-promoted functional recovery after brain injury. The symbol (+) in the figure indicates positive effects.