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Neuroprotective Effect of Granulocyte-Colony Stimulating Factor

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1. ABSTRACT

Granulocyte-colony stimulating factor (G-CSF) is a growth factor which stimulates proliferation, differentiation, and survival of hematopoietic progenitor cells. G-CSF is being used extensively in clinical practice to accelerate recovery of patients from neutropenia after cytotoxic therapy. However, growing evidences have suggested that G-CSF has important non-hematopoietic functions in central nervous system. Recent studies have shown the presence of G-CSF/G-CSF-receptor (G-CSFR) system in the brain, and their roles in neuroprotection and neural tissue repair as well as improvement in functional recovery. The increased expression of G-CSF/G-CSFR on neurons subjected to hypoxia provides evidence that G-CSF may have an autrocrine protective signaling mechanism in response to neural injury. G-CSF exerts neuroprotective actions through the inhibition of apoptosis and inflammation and the stimulation of neurogenesis. Moreover, G-CSF has been shown to mobilize bone marrow stem cells into the injured brain improving neural plasticity. In this review, we summarize some of the recent studies on G-CSF and the corresponding signal transduction pathways regulated by G-CSF in neuroprotection.

2. INTRODUCTION

Each year about 700,000 people experience a new or recurrent stroke which is the leading cause of adult disability and remains the third most common cause of death in the United States (1). For the last two decades, a great deal of attention has been paid to identify the pathophysiological pathways leading to neural death in stroke and to design neuroprotectant agents to modulate these pathways. Although numerous agents have been found to reduce infarct size and improve clinical outcome in experimental models, the clinical use of these neuroprotective agents has been hampered by toxicity of the compounds. Recombinant tissue plasminogen activator (rt-PA) remains the only approved agent for acute stroke management in humans. However, its narrow therapeutic window and potential side effects including increased risk of intracerebral hemorrhage and neurotoxicity limit the efficacy of rt-PA (2-4).

In recent years, several independent research groups have reported neuroprotective properties of growth factors such as erythropoietin (EPO), insulin-like growth factor, basic fibroblast growth factor, brain-derived neurotrophic factor (BDNF), and granulocyte-colony

stimulating factor (G-CSF) in central nervous system diseases such as stroke, neurotrauma, neuroinflammatory, and neurodegenerative diseases using experimental animal models (5-12). G-CSF, a member of growth factor family, not only stimulates the development of committed progenitor cells to neutrophils but also modulates the neutrophil functions and their distribution in the body (13). Furthermore, like other cytokines, it also has trophic effects on the different cell types including neuronal cells (14). This could be of interest, because neurotrophic factors are not only essential for the survival and differentiation of normally developing neurons, but they also play important roles in the protection and recovery of mature neurons under pathologic conditions (15). Although there is evidence from recent experimental studies that administration of G-CSF is neuroprotective (16-20), the precise mechanisms of the neuroprotective effect of G-CSF are not entirely explored.

In this article, we review the biological properties and clinical applications of G-CSF, and the possible role and novel mechanisms of G-CSF as a potential therapeutic agent for neuroprotection.

3. G-CSF AND ITS RECEPTOR

G-CSF is a glycoprotein with a molecular mass of 19 kDa and is structurally characterized by four anti-parallel alpha-helices (21). G-CSF is produced by a variety of cells including bone marrow stromal cells, endothelial cells, macrophages, fibroblasts, and astrocytes in response to specific stimulation (13, 22, 23). Human G-CSF is encoded by a single gene that is located on chromosome 17 q11-12 (24, 25).

G-CSF binds to its specific receptor and stimulates the proliferation and differentiation of neutrophilic progenitor cells. The G-CSF receptor (G-CSFR) is a type I membrane protein and has a composite structure consisting of an immunoglobulin-like domain, a cytokine receptor-homologous domain and three fibronectin type III domains in the extracellular region (26). G-CSFR is expressed not only on a variety of hematopoietic cells including neutrophils, and their precursors, monocytes, platelets, lymphocytes, and leukemia cells, but also on non-hematopoietic cells such as endothelial cells, neurons and glial cells (20, 27-32).

G-CSFR activates a variety of intracellular cascades, including the Janus kinase (JAK)/signal transducer and activator of transcription (STAT), the Ras/mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3-K)/protein kinase B (PKB) (also known as Akt) (33-36). Activation of these cascades subsequently activates their downstream substrates and affects target genes which mediate proliferation, differentiation, and survival of hematopoietic cells (37). These signaling cascades are summarized in Figure 1.

3.1. G-CSF and G-CSFR in the brain

G-CSF receptor and its ligand have been shown to be expressed by neurons in a variety of brain regions including pyramidal cells in cortical layers (particularly in layers II and V), Purkinje cells in the cerebellum, subventricular zone (SVZ) and cerebellar nuclei in rats. G-CSF positive cells in CA3 region of the hippocampus, subgranular zone and hilus of the dentate gyrus, entorhinal cortex, and olfactory bulb have been identified (12). Moreover, G-CSF receptor expression has shown in the frontal cortex of human brain by postmortem studies (12).

G-CSF and its receptor are co-expressed in neurons in the rodent central nervous system and are upregulated in the ipsilateral forebrain hemisphere after 2 hours occlusion of the middle cerebral artery (MCAO) and reperfusion (12). Similarly, Kleinschnitz *et al.* showed that 4 hours after permanent MCAO, G-CSF mRNA levels massively increases compared to normal cortex and decreases after 2 days to its control levels (38). The authors pointed out that the increase in G-CSF mRNA expression is not only within the ischemic lesions but also in the nonischemic frontal cortex after photothrombosis model of focal cerebral ischemia (38). Taken together, current evidences suggest that G-CSF may have an autrocrine protective signaling mechanism in response to neural injury. Similar mechanisms have been suggested for other growth factors, especially EPO which have been extensively reviewed by others (39, 40).

4. CLINICAL APPLICATIONS OF G-CSF

4.1. G-CSF in general clinical use

G-CSF, specifically Filgrastim (r-metHuG-CSF), a genetically engineered drug was approved by FDA on February 21, 1991 to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs associated with a significant incidence of severe neutropenia with fever. During the last decade, G-CSF has found wide spread use in reducing the duration of febrile neutropenia in cancer patients treated with chemotherapy. G-CSF is also being used clinically to facilitate hematopoietic recovery after bone marrow transplantation, the mobilization of peripheral blood progenitor cells in healthy donors and in the treatment of severe congenital neutropenia (41-44).

The role of G-CSF treatment in variety of other conditions is still matter of discussion. There is evidence from clinical studies that administration of G-CSF is safe, beneficial, and well tolerated for the prevention or treatment of infections in patients with non-neutropenic infections, patients undergoing surgery, patients with human immunodeficiency virus, and patients with diabetic foot infections (45-50). On the other hand, controlled trials and systematic reviews have concluded that there is no current evidence supporting the routine use of G-CSF in the treatment of pneumonia, in patients undergoing surgery, for treating or preventing neonatal infections, and in the treatment of diabetic foot infections (51-54).

4.2. G-CSF treatment in patients with cerebral injuries

The prophylactic administration of G-CSF against severe infections in critically ill patients with

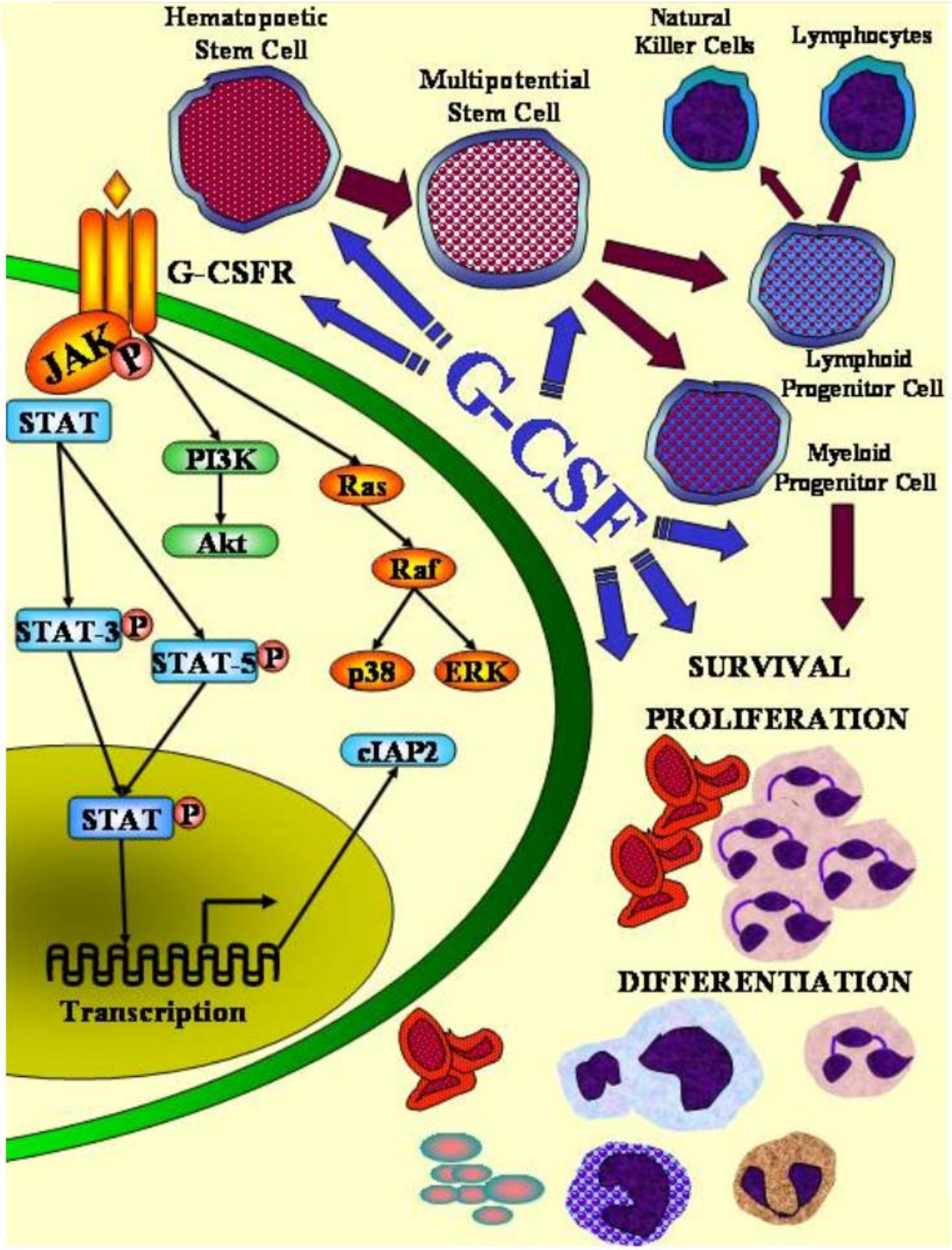

Figure 1. Figure shows the hematopoetic actions of Granulocyte-Colony Stimulating Factor (G-CSF). G-CSF plays an important role in the survival, proliferation and differentiation of the hematopoetic cells. The figure also depicts the signaling pathways suggested in the existing literature for the G-CSF mediated actions. G-CSFR activates Janus kinase (JAK)/signal transducer and activator of transcription (STAT), the Ras/mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3-K)/Akt pathways. Activation of JAK2/STAT3 leads to increased expression of cellular inhibitor of apoptosis protein2 (cIAP2) in human neutrophils resulting in their survival. Activation of STAT3 by G-CSF has also been linked to myeloid cell differentiation. STAT5, however, seems to be involved in G-CSF-dependent cell proliferation.

cerebral injuries is controversial. The primary outcome of studies is a reduction in frequency of bacteremia and in life-threatening infections in patients with cerebral injuries. For the first time, Heard *et al.* conducted a randomized, placebo-controlled, double-blind, multi-center, phase II study to determine whether the use of prophylactic recombinant human G-CSF reduces the frequency of nosocomial infections in patients with either acute traumatic brain injury or cerebral hemorrhage (55). The primary efficacy end points of this study were the frequency of nosocomial infections, increase in absolute neutrophil count, and the safety of G-CSF. Secondary efficacy end points included serum G-CSF concentrations, 28 day survival, and duration of hospitalization, antibiotic use, intensive care unit stay, and mechanical ventilation. Authors reported that G-CSF increases absolute neutrophil counts and markedly reduces the frequency of bacteremia in a dose-dependent fashion in critically ill and intubated patients (55). They concluded that phase III trial is warranted to confirm these results. However, the authors did not report neurological outcomes and cerebral physiological variables of the patients. Also, the study was criticized by Whalen *et al.* because the systemic administration of G-CSF to patients with traumatic brain injury may carry potential risk of increased acute inflammatory reactions in the injured brain due to excessive accumulation of neutrophils (56). Whalen *et al.* have suggested that it was premature to use G-CSF in humans with TBI until animal studies have demonstrated G-CSF effects on brain injury after trauma or stroke (56).

Combined therapy with high-dose barbiturates and mild hypothermia has been used widely for patients with severe head injuries to decrease their intracranial pressure (ICP). However, both therapies alone may result in a decrease in leukocytes and suppression of neutrophil functions which increase susceptibility to infectious complications (57, 58). Ishikawa *et al.* have used G-CSF to ameliorate life-threatening infections in patients with severe head injury who were treated with combined therapy involving high-dose barbiturates and mild hypothermia (59). Authors studied the daily changes in total leukocyte count, leukocyte differentiation, C-reactive protein, respiratory index (RI), and ICP. Patients treated with G-CSF showed significant improvement in the RI which reflects the recovery from pneumonia. Authors reported that ICP was not elevated by G-CSF administration, and a significant improvement was achieved in survival rate. Moreover, G-CSF treatment resulted in decrease of blood IL-6 concentrations of patients. Although previous studies that have attempting to correlate IL-6 production with patient outcome following TBI have produced inconsistent results, Arand *et al.* suggested that elevated IL-6 in the serum or CSF is associated with poor outcome (60). Ishikawa *et al.* concluded that G-CSF treatment ameliorates life-threatening infections in patients with severe head injury who were treated with combined therapy involving high-dose barbiturates and mild hypothermia without causing lung injury or increasing brain swelling (59). However, this was not a prospective study and the number of patients was small to conclude whether the use of G-CSF in brain injured patients is a safe prophylactic agent against severe infections.

In summary, the therapeutic potential of prophylactic G-CSF therapy in critical ill patients, and G-CSF administration for the prevention or treatment of infections in non-neutropenic patients are currently unproven and further controlled trials will be necessary to clarify these issues.

5. POTENTIAL NEUROPROTECTIVE PROPERTIES OF G-CSF

There is growing number of studies about the neuroprotective properties of growth factors in the last decade. Although G-CSF has been used for many years in clinical practice and is a well known growth factor, there are only a few experimental studies exploring the neuroprotective effects of G-CSF. These studies are summarized in Table 1.

5.1. G-CSF, inflammation, and apoptosis

It is well established that the inflammation, in response to brain injury, involves infiltration of neutrophils and monocytes/macrophages into the injured brain parenchyma and activation of resident brain cells which are capable of generating inflammatory mediators (61, 62). Although there is not a clear cause-effect relationship between neutrophil recruitment and CNS pathogenesis (63), it is well known that neutrophil activation results in release of proteolytic enzymes and generation of free oxygen radicals. Excessive generation of free oxygen radicals causes DNA damage, lipid peroxidation and inactivation of proteins and finally leads to severe tissue injury (64-66). Free oxygen radicals also contribute to the breakdown of the blood-brain barrier (BBB) and brain edema (62, 67). It could be of interest because G-CSF administration increases absolute neutrophil count in peripheral blood which may result in acute inflammatory reactions in the injured brain. In support of this, it has been reported that *in vivo* chloroquine or colchicine treatment that reduce the number of mononuclear phagocytes in damaged brain, help to block reactive astrogliosis and neovascularization, and slow the rate of debris clearance from sites of traumatic injury (68). For the first time, Whalen *et al.* evaluated the effect of G-CSF-induced neutrophilia on BBB permeability and brain edema after traumatic brain injury in a rat model (69). They have demonstrated that subcutaneous administration of G-CSF (25 µg/kg every 12 hrs for five doses, with the last dose administrated immediately before cortical impact injury (CCI)) resulted in increased systemic absolute neutrophil count (ANC) at the time of controlled CCI which correlated with BBB damage in the injured hemisphere 24 hours later. However, no difference in brain edema and hemispheric neutrophil accumulation were reported between control and G-CSF treated rats. They have suggested that the ability of G-CSF-stimulated neutrophils to migrate into injured tissue may be impaired (69). Recently, Park *et al.* have reported that intra-peritoneal G-CSF administration after intracerebral hemorrhage reduces brain edema and inflammation in a rat model (17). However, they also

Table 1. Previous experimental studies about neuroprotective effect of G-CSF treatment after CNS injuries

Species	Model	Results	Author	Year	Ref.
Rat	Pretreatment TBI	No increase in brain edema and posttraumatic brain neutrophil accumulation; Increase in BBB damage;	Whalen M. J., *et al.*	2000	69
Mice	EAE	Limitation of demyelination and inflammation; Clinical score improvement; Reduction of pro-inflammatory cytokines;	Zavala F., *et al.*	2002	74
Mice	MCAO	Improving survival rate; Reduction of infarct volume;	Six I., *et al.*	2003	18
Rat	MCAO Cell Culture	Reduction of infarct volume; Reduction of inflammation; Protection against excitotoxicity;	Schabitz W. R., *et al.*	2003	20
Mice	TBI	Small functional effect on functional outcome; No effect on histopathology or motor outcome;	Sheibani N., *et al.*	2004	19
Rat	MCAO	Neurological improvement; Reduction of infarct volume; Improving neural plasticity and vascularization;	Shyu W. C., *et al.*	2004	16
Rat	ICH	Neurological improvement; Reduction of edema, inflammation and perihematomal cell death;	Park H. K., *et al.*	2005	17
Mice	MCAO	Neurological improvement; Reduction of infarct volume;	Gibson C. L., *et al.*	2005	11
Rat	MCAO CCCA/MCAO Cell culture	Neurological improvement; Reduction of infarct volume; Induce neurogenesis;	Schneider A., *et al.*	2005	12

TBI; traumatic brain injury, EAE; experimental allergic encephalomyelitis, MCAo; middle cerebral artery occlusion, ICH; intracerebral hemorrhage, CCCA/MCAo; combined common carotid artery/ middle cerebral artery occlusion, BBB; blood brain barier, Ref: Reference

showed that G-CSF treatment also decreases the BBB permeability after injury leading to contradictory results (17). The neuroprotective effect of G-CSF in neurons was suggested by Schabitz *et al.*, by demonstrating that intravenous administration of G-CSF after focal cerebral ischemia reduced the volume of infarction and the number of infiltrated neutrophils into the ischemic hemisphere 24 hours later. Furthermore, neuroprotective effect of G-CSF is further supported by the finding that G-CSF protects cerebellar granule cells exposed to glutamate *in vitro* (20).

Peripherally derived leukocytes not only release proteolytic enzymes and free oxygen radicals but also produce and secrete cytokines including tumor necrosis-alpha (TNF-alpha) and interleukin-1-beta (IL-1beta) which mediate BBB injury and enhance the migration of blood leucocytes into the injured tissue by modulating the expressions of various surface antigens (62, 70, 71). The possible mechanisms of G-CSF treatment on the prevention of BBB damage may be through its ability to decrease the levels of proinflammatory cytokines. A series of reports have demonstrated that G-CSF displays immunoregulatory properties through interaction with its receptor. It has been shown that G-CSF treatment protects rodents against lipopolysaccharide (LPS)-induced lethal toxicity by suppressing systemic TNF-alpha production *in vivo* (72). Such reduced cytokine production as well as by an increase in production of anti-inflammatory counterregulatory molecules has also been shown in peripheral blood leukocytes from G-CSF-treated healthy volunteers (73). Also, G-CSF reduces the T cell infiltration and inflammation within the CNS in experimental allergic encephalomyelitis by reducing interferon-gamma (IFN-gamma) and increasing IL-4 and TGF-beta1 levels, and with a reduction of systemic and lymphocyte TNF-alpha production (74). Nishiki *et al.* have suggested that G-CSF inhibits LPS-induced TNF-alpha production in human monocytes through selective activation of JAK2/STAT3 pathways (75).

G-CSF activates JAK2 in turn activates a transcription factor, STAT3, triggering a signaling transduction cascade that leads to increase in cellular inhibitor of apoptosis protein2 (cIAP2) expression in human neutrophils resulting in their survival (76) (Figure 1). Activation of STAT3 by G-CSF has also been linked to myeloid cell differentiation (77). However, STAT5 seems to be involved in G-CSF-dependent cell proliferation (78). Also, G-CSF activation of JAK2-STAT3 pathway and the resulting induction of antiapoptotic proteins and inhibition of apoptotic death of cardiomyocytes in the infarcted hearts have been recently reported by Harada *et al.* (79). Schabitz *et al.* showed for the first time that G-CSF treatment results in increased STAT3 expression in the penumbra of the infarction after focal cerebral ischemia in rats and they have suggested that upregulation of STAT3 in neurons may mediate the antiapoptotic effects of G-CSF (20). Antiapoptotic effect of G-CSF in neural tissue was also pointed by Park *et al.* by demonstrating reduced number of TUNEL positive neurons in the penumbra following G-CSF treatment after intracerebral hemorrhage in rats (17).

More recently, G-CSF has been reported to protect cortical neurons *in vitro* against camptothecin-induced and NO-induced apoptosis by reducing caspase-3 and poly(ADP-ribose) polymerase (PARP) cleavage (12). Authors showed that G-CSF exposure results in rapid STAT3 phosphorylation by JAK2 kinase in neurons, which was inhibited by AG 490, a specific inhibitor of JAK2. The authors further reported that G-CSF leads to increase of protein levels of the antiapoptotic STAT3 target Bcl-X_L (Figure 2) (12). However, determination of activation levels of the extracellular signal-regulated kinase (ERK) family showed that while ERK1/2 was transiently and weakly

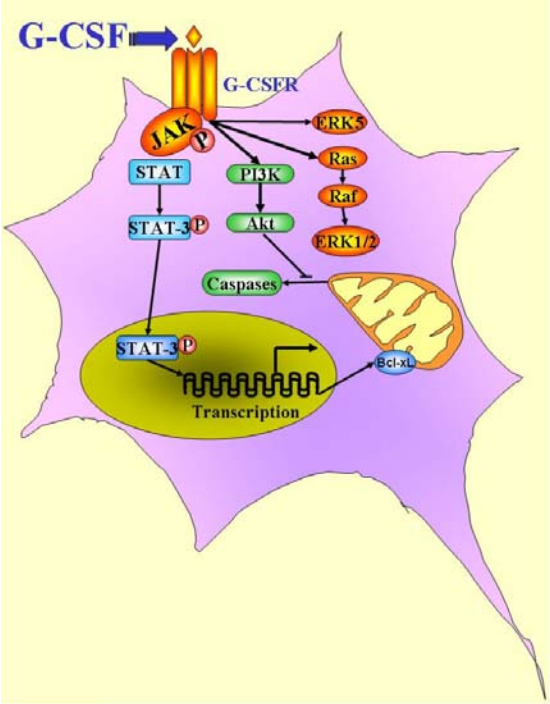

Figure 2. Figure shows the signaling pathways implicated in G-CSF mediated neuronal survival. Activation of the extracellular signal-regulated kinase (ERK) family enhances neuronal survival. PI3-K/Akt and STAT3 signaling pathways when activated prevent apoptotic cell death by inhibiting activation of caspases and increasing antiapoptotic protein members such as Bcl-xL.

ctivated by G-CSF, ERK5 kinase was strongly activated in cultured neurons from rat cortex (12). ERK5, also known as big MAP kinase 1 (BMK1), is a MAPK member whose biological role is largely undefined. However, it has been suggested that ERK5 activation enhances neuronal survival (80, 81). Schneider *et al.* also showed the activation of PI3-K/Phosphoinositide-dependent kinase (PDK)/Akt signaling pathway by G-CSF in cortical neurons *in vitro*. They suggested that anti-apoptotic action of G-CSF on neurons mediated at least partially by the PI3-K/Akt pathway (12) (Figure 2). It is well established that Akt, a downstream target of PI3-K, is a critical anti-apoptotic factor in controlling the balance between survival and apoptosis in multiple cell systems including neurons by several mechanisms such as by phosphorylating Bcl-2-associated death protein (BAD) and caspase-9, and inducing Bcl-2 expression (82-86). PDK has also been shown to phosphorylate and activate Akt to promote cell survival (87).

There is strong evidence from previous studies that G-CSF exerts antiapoptotic effect on many hematopoietic cell lines by triggering various signal pathways. G-CSF inhibits spontaneous cytochrome *c* release and mitochondria dependent apoptosis of myelodysplastic syndrome hematopoietic progenitors (88). It has been reported that G-CSF-induced survival of neutrophils associates with inhibition of cleavage of Bid, Bid/Bax translocation to the mitochondria, and prevention of subsequent release of the proapoptotic mitochondrial constituents. Moreover, G-CSF blocks both processing of the initiator caspase-8 and caspase-9 and the executioner caspase-3 and their specific enzymatic activities in apoptotic neutrophils (89). Furthermore, G-CSF inhibits the processing of proIL-1beta and the release of mature IL-1beta in LPS-stimulated whole blood via the inhibition of caspase-1 which is also known as interleukin-converting enzyme (90). It has also been shown that mice deficient in interleukin-1 converting enzyme are resistant to neonatal hypoxic-ischemic brain damage (91). These mechanisms suggest probable mechanisms for the anti-apoptotic effects of G-CSF treatment on hematopoietic cell lines. However, it needs to be studied whether G-CSF maintains viability of neuronal cells by similar mechanisms. The precise mechanism by which G-CSF exerts anti-apoptotic function in the neural tissue *in vivo* is not well understood.

5.2. G-CSF, hematopoietic stem cell mobilization, and brain repair

There is a growing interest to restore brain function after stroke by transplanting stem cells. Various stem cells especially porcine fetal cells, neural stem cells (NSCs), and bone marrow stem cells (BMCs) currently are under investigation by various independent research groups (92-95).

Recent studies have focused on the potential plasticity of bone marrow stem cells (BMCs) after stroke. There are two populations of bone marrow stem cells: hematopoietic stem cells (HSCs), which can differentiate into every type of mature blood cells; and mesenchymal stem cells (MSCs), which can differentiate into adipose, muscle, bone, cartilage, endothelium, hepatocyte, glia, and neuron under appropriate stimuli *in vitro* and *in vivo* (96-104). However, some controversy still exists about differentiation of BMCs into neural tissues (105-107).

In previous studies, it has been reported that after direct intrastriatal, intracarotid, and intravenous delivery, MSCs migrate to the ischemic area, survive and differentiate into neuronal and glial cell types, and improve functional recovery after experimental stroke (92-94). The migration of MSCs is supported by evidence that Y chromosome-positive neuron-like cells are seen in postmortem brain samples from females who had received bone marrow transplants from male donors in humans (95).

Although various studies attributed the beneficial results of BMC treatment after stroke to possible neuronal plasticity, the precise mechanisms of the functional benefits still remains unclear. Several studies have showed functional benefits may result either from enhanced neurogenesis or from a favorable impact of stem cells or their released cytokines which stimulate angiogenesis and reduce cell death. It has been shown that treatment of stroke with human MSCs enhances angiogenesis in the host brain which is mediated by increase in levels of endogenous rat vascular endothelial growth factor (VEGF) and VEGF-

receptor 2 (108). Other studies have shown reduction of apoptosis, increase in BDNF and nerve growth factor in the penumbral zone of the lesion, and the proliferation of endogenous cells in the subventricular zone after administration of human MSCs in rats (109). Moreover, it has been suggested that intrastriatal transplantation of mouse MSCs restores cerebral blood flow and BBB to normal levels, elevates expression levels of neurotrophic factors including activin A, the glial cell line-derived neurotrophic factor, and transforming growth factor-beta 1 and 2 (110). So, these mechanisms of recovery may also be due to the release of trophic factors into the damaged area rather than neuronal differentiation and implant integration to the injured ischemic site.

Recently, Bang *et al.* have examined the feasibility, efficacy, and safety of cell therapy using culture-expanded autologous MSCs in patients with ischemic stroke (111). They have reported that intravenous infusion of $1x10^8$ autologous MSCs to patients, more than 1 month after the onset of stroke symptoms, improves outcome (111). However, they stress the need of future double-blind studies with larger cohorts to reach a definitive conclusion regarding the efficacy of MSC therapy (111).

It is well known that administration of G-CSF mobilizes HSCs from bone marrow into peripheral blood (13). G-CSF has been shown to result in significant decrease in infarct volume and enhance survival rate after focal cerebral ischemia in mice, and these benefits have been suggested to be mediated by mobilization of autologous HSCs into circulation and their contribution to brain repair (18). Moreover, Shyu *et al.* showed that G-CSF treatment increases $BrdU^+$ cells coexpressing the neuronal phenotypes of $Neu\text{-}N^+$ and microtubule-associated protein-2 $(MAP\text{-}2)^+$ cells as well as the glial phenotype of $GFAP^+$ cells in the ischemic cortical areas of G-CSF-treated rats after focal cerebral ischemia in rats (16). They have suggested that G-CSF treatment enhances translocation of HSCs into ischemic brain, and significantly improves lesion repair by improving neuronal plasticity and vascularization (16). Interestingly, they have reported that neutralization of CXC chemokine receptor 4 (CXCR4) by its specific antibody results in a slight reduction in infarct volume in G-CSF-treated rats (16).

The chemokine receptor CXCR4 is expressed in cells of both the immune and the central nervous systems and can mediate migration of resting leukocytes and hematopoietic progenitors in response to its ligand, stromal cell-derived factor-1 (SDF-1). SDF-1, the strong chemoattractant was also shown to be crucial for cerebellar development (112, 113). Moreover, it has been suggested that SDF-1/CXCR4 system may control cerebral infiltration of CXCR4-carrying leukocytes during cerebral ischemia, and contribute to ischemia-induced neuronal plasticity (114). SDF-1 also play pivotal role in the migration of NSCs toward the ischemic core and penumbra, and enhances proliferation, promotes chain migration and activates intracellular molecular pathways mediating engagement (115). Both microglial cells and astrocytes in humans can express chemokine receptors including CCR4, CCR5, CCR6, CXCR2, and CXCR4 and activation of the cells with TNF-alpha and IFN-gamma changes the expression levels of these chemokine receptors (116, 117). CXCR4-mediated signaling in astroglioma cells also provides pathway for these cells to express chemokines involved in angiogenesis and inflammation (118). Taken together these data, a better understanding of this complex dynamic and its relationships with G-CSF may permit us to understand precise mechanisms of G-CSF related functional recovery after stroke.

5.3. G-CSF and neurogenesis

The subgranular zone of dentate gyrus (DG) in the hippocampus and subventricular zone (SVZ) of the lateral ventricle are the main areas of the adult brain that undergo neurogenesis and gliogenesis (119-121). The SVZ in the forebrain is the largest source of neural stem cells (NSCs) and progenitor cells which can differentiate into neurons, astrocytes, and oligodendrocytes (120, 122). There is accumulating evidence indicating that this neurogenerative capacity of the adult CNS could open novel therapeutic approaches to neurodegenerative diseases based on the use of NSC transplantation. Previous *in vivo* experiments showed that transplanted human NSCs survive, migrate, and differentiate in host brain and promote functional recovery after intracerebral hemorrhage and cerebral ischemia (123-125). Endogenous NSCs sustain neurogenesis and gliogenesis in response to several different injuries including ischemic and traumatic brain injury (126-128).

It has been reported that outcome from ischemia appears to be worse when neurogenesis is inhibited by irradiation in gerbils (129). However, augmentation of this self-repair phenomenon by exogenous agents can further enhance neurogenesis and might also have therapeutic applications after CNS disorders. Enhanced neurogenesis with growth factors such as EPO or heparin-binding epidermal growth factor-like growth factor (HB-EGF) have been reported to decrease infarct size and improve neurological functions after stroke in rats (130, 131).

More recently, G-CSF has been shown to have functional role in differentiation of adult rat NSCs both *in vitro* and *in vivo* (12). Analysis of G-CSF responsiveness in the cultured NSCs from the rat SVZ or hippocampal region indicates that G-CSF exposure results mainly in increase in the population of cells expressing mature neural markers such as beta-III-tubulin, neuron-specific enolase (NSE), and MAP-2 without elevating the number of immature stem cells (12). Schneider *et al.* found that peripheral infusion of G-CSF enhances recruitment of progenitor cells from the lateral ventricular wall into the ischemic area of the neocortex in rat (12). Moreover, authors reported that G-CSF increases hippocampal neurogenesis not only in ischemic animals, but also in the intact, non-ischemic rat. Based on this evidence, they argued that G-CSF may enhance structural repair and function even in healthy subjects or at long intervals after stroke (12).

The intracellular mechanisms that regulate neurogenesis both in normal and ischemic conditions remain unclear. Increased cyclic guanosine monophosphate (cGMP), a molecular messenger involved in regulation of cellular proliferation, has been reported to enhance neurogenesis (132). Recently, Wang *et al.* showed that PI3-K/Akt pathway mediates cGMP enhanced proliferation of adult progenitor cells derived from the SVZ of the rat (133). Moreover, it has been suggested that stroke-enhanced neuroblast migration is independent of cell proliferation and survival, and PI3-K/Akt signal transduction pathway mediates neuroblast migration after stroke (134). The activation of PI3-K/Akt signaling pathway by G-CSF has been shown in cortical neurons *in vitro* (12). The anti-apoptotic effect of G-CSF on neurons is dependent on activation of PI3-K/Akt signaling pathway. It can be further hypothesized that G-CSF may also augment neurogenesis and neuroblast migration by activation of PI3-K/Akt signaling pathway.

It has been shown that CXCR4 is expressed in rat and human neural progenitor cells (135). The CXCR4/SDF-1 system is important in mediating specific migration of neural progenitor cells to the site of ischemic damaged neurons (115, 136). As discussed previously, neutralization of CXCR4 by its specific antibody has been reported to reduce neuroprotective effect of G-CSF in rats after stroke (16). Thus, it can be hypothesized that CXCR4/SDF-1 system may play important role in G-CSF induced neurogenesis. Additional studies however will be necessary to clarify this issue.

It is well known that the regulation of adult neurogenesis and the maintenance of stem cell renewal are modulated by various regulatory mechanisms including growth factors. Although the ability of G-CSF to stimulate the neurogenesis shows a lot of promise, additional studies are required for determining the molecular basis of this effect.

6. SUMMARY AND PERSPECTIVE

An increasing amount of data suggests a neuroprotective role for G-CSF. The molecular mechanisms of neuroprotective effect of G-CSF especially under *in vivo* settings still remain unclear. However, the neuroprotective property of G-CSF may be due, at least in part, to the mobilization of BMCs to the injured brain. However, the precise mechanism of BMCs-mediated brain repair and/or neuroprotection after stroke is still under investigation. Furthermore, G-CSF has anti-apoptotic, anti-inflammatory, immunomodulatory, and neurogenesis-inducing effects which provide other potential mechanisms that may be responsible for neuroprotection and long term benefits.

Neuroprotective effects of G-CSF however, should be tested in multiple animal models in different species with different time points, different doses and application routes to confirm results. Also, the use of G-CSF in conjunction with rt-PA should be tested for safety before starting clinical trials in humans.

7. ACKNOWLEDGEMENT

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