A Novel Neuroprotectant Granulocyte-Colony Stimulating Factor

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Background and Purpose—Granulocyte-colony stimulating factor (G-CSF) is a growth factor that orchestrates the proliferation, differentiation, and survival of hematopoietic progenitor cells. It has been used for many years in clinical practice to accelerate the recovery of patients from neutropenia after cytotoxic therapy. However, there is a growing body of evidence from experimental studies suggesting that G-CSF also has important nonhematopoietic functions in the central nervous system.

Summary of Review—The presence of the G-CSF/G-CSF–receptor system in the brain and its role in neuroprotection and neural tissue repair has been investigated in many recent studies. The neuroprotective actions of G-CSF have mainly been attributed to its anti-inflammatory and antiapoptotic effects. Furthermore, it induces neurogenesis and angiogenesis and improves functional recovery. In this review, we summarize the role of G-CSF and the corresponding signal transduction pathways regulated by G-CSF in neuroprotection.

Conclusions—Much additional work is needed to better understand the precise mechanisms of G-CSF–induced neuroprotection. However, there is emerging data suggesting that G-CSF is a potential new agent for neuroprotection. (Stroke. 2006;37:1123-1128.)

Key Words: granulocyte-colony stimulating factor / growth factor / neuroprotection / stem cell / stroke

The use of growth factors as neuroprotective agents for various types of neurological disorders has been under investigation for many years by different research groups. Previous studies using experimental animal models have demonstrated the neuroprotective effects of growth factors, such as erythropoietin (EPO), and brain-derived neurotrophic factors in central nervous system diseases, such as stroke, neurotrauma, and neurodegenerative diseases.1–3 Of note, some growth factors, such as EPO, have been used in clinical practice. EPO has been reported to be a well-tolerated agent in acute ischemic stroke and is associated with an improved clinical outcome in humans.4

Granulocyte-colony stimulating factor (G-CSF), a member of the growth factor family, mainly stimulates the development of committed progenitor cells to neutrophils and also modulates neutrophil actions and their distribution in the body.5 It also has trophic effects on the different cell types, including neuronal cells.6 Recent experimental studies have shown that the administration of G-CSF is neuroprotective7–16; however, the precise mechanisms of the neuroprotective effect of G-CSF are not entirely known. Therefore, we have summarized the biological properties, clinical applications, role, and novel mechanisms of G-CSF as a potential therapeutic agent for neuroprotection.

Structure of G-CSF and Its Receptor Structurally, G-CSF is a glycoprotein consisting of 4 antiparallel \( \alpha \) helices with a molecular mass of 19 kDa.17 A variety of cells, including bone marrow stromal cells, fibroblasts, macrophages, endothelial cells, and astrocytes, can produce G-CSF in response to a variety of stimulants.5,18,19 The proliferation and differentiation of neutrophilic progenitor cells is largely dependent on the binding of G-CSF to its specific receptor (G-CSFR). G-CSFR is expressed on a variety of hematopoietic cells and also on nonhematopoietic cells, such as neurons, endothelial cells, and glial cells.12,20–25 The G-CSFR has a composite structure consisting of an immunoglobulin-like domain, a cytokine receptor–homologous domain, and 3 fibronectin type III domains in the extracellular region. However, G-CSFR does not have its intracellular kinase domain as do other hematopoietin receptor superfamiliy members. The cytoplasmic domain of G-CSFR consists of 3 amino acid sequences (boxes 1 to 3), which are critical for the signal transduction of the G-CSFR.26

G-CSF mediates proliferation, differentiation, and survival of hematopoietic cells mainly by the activation of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT), the Ras/mitogen-activated protein (MAP) kinase, and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (also known as Akt) signaling pathways.27–31
Clinical Applications of G-CSF

On the February 21, 1991, r-metHuG-CSF (Filgrastim), a genetically engineered drug, was approved by the US Food and Drug Administration to decrease the incidence of infection, as manifested by febrile neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs. G-CSF is also being used clinically to facilitate hematopoietic recovery after bone marrow transplantation, to mobilize peripheral blood progenitor cells in healthy donors, and to treat severe congenital neutropenia.32–35 Available data suggest that there is little doubt that G-CSF is a safe agent for use in the human population.

G-CSF as a Neuroprotectant

G-CSF and G-CSFR in the Brain

The expression of G-CSFR in neuronal cells has been shown in a variety of brain regions, such as pyramidal cells in the cortical layers, Purkinje cells in the cerebellum, subventricular zone (SVZ), and cerebellar nuclei in rats. Cells in the CA3 region of the hippocampus, subgranular zone and hilus of the dentate gyrus, entorhinal cortex, and olfactory bulb, positively staining for G-CSF have also been identified previously.13 Furthermore, in a postmortem study, G-CSFR expression has been demonstrated in the frontal cortex of the human brain.13 Similarly, our experiments showed localization of G-CSFR on neural cells in rat brain and spinal cord samples (Figure 1).

Recent evidence suggests that G-CSF may have an autocrine protective signaling mechanism in response to neural injury in the central nervous system (CNS). Coexpression and upregulation of G-CSF and its receptor in neurons after middle cerebral artery occlusion and reperfusion injury have been reported in the rodent central nervous system.13 Kleinschnitz et al16 showed that permanent middle cerebral artery occlusion resulted in increasing levels of G-CSF mRNA compared with the normal cortex after 4 hours and decreased after 2 days to baseline. Furthermore, an increase in G-CSF mRNA expression was not only seen in the ischemic lesion but also in the nonischemic frontal cortex after a photothermal model of focal cerebral ischemia.36

G-CSF Has an Anti-Inflammatory Effect on CNS

Inflammation, in response to brain injury, involves infiltration of inflammatory cells into the injured brain parenchyma and activation of resident brain cells, which are capable of generating inflammatory mediators that have been extensively reviewed by others.37,38 Whalen et al39 evaluated the effect of G-CSF–induced neutrophilia on blood–brain barrier (BBB) permeability and brain edema after traumatic brain injury in a rat model. They demonstrated that G-CSF treatment resulted in an increased systemic absolute neutrophil count, which correlated with BBB damage in the injured hemisphere. However, no difference in brain edema and hemispheric neutrophil accumulation were reported between the control and G-CSF–treated rats. As a result Whalen et al39 suggested that the ability of G-CSF–stimulated neutrophils to migrate into injured tissue may be impaired. However, Park et al40 conversely reported that intraperitoneal G-CSF administration after intracerebral hemorrhage reduced brain edema and inflammation and decreased the BBB permeability in the rat model. These findings are additionally supported by many others in different stroke models. Gibson et al7 showed using an MRI study that G-CSF significantly reduces the amount of edematous brain tissue after cerebral ischemia in mice. Also, Lee et al10 reported that G-CSF reduces BBB disruption and suggested that this effect may be mediated by reduced inflammation and cellular damage in the acute phase of brain injury. Furthermore, G-CSF administration is also associated with a marked decrease in the number of infiltrated neutrophils within the hemisphere undergoing infarction experimentally.10,12

It is well established that peripherally derived leukocytes release proteolytic enzymes and free-oxygen radicals. They can also produce and secrete cytokines, including tumor necrosis factor (TNF)-α and interleukin-(IL)-1β, which mediate BBB injury and enhance the migration of blood leukocytes into the injured tissue.37,40,41 It can be hypothesized that 1 of the possible mechanisms of action of G-CSF in the prevention of BBB breakdown is its ability to attenuate the inflammatory response. For example, inflammation and its associated T-cell infiltration into the CNS was shown to be reduced by G-CSF in an experimental allergic encephalomyelitis model.42 The migration of T cells to the CNS is under the control of chemokines, including macrophage inflammatory protein-1α and macrophage chemoattractant protein-1. G-CSF reduces the macrophage inflammatory protein-1α: macrophage chemoattractant protein-1 chemokine protein ratio after experimental allergic encephalomyelitis, which reduces T-cell migration to the CNS.42 This was accomplished by increasing IL-4 and TGF-β1 levels and by reducing interferon-γ, as well as systemic and lymphocytic TNF-α production.42 On the other hand, Gibson et al7 reported that G-CSF treatment significantly suppresses IL-1β mRNA up-regulation after cerebral ischemia in mice while having no effect on TNF-α or inducible NO synthase (iNOS) mRNA expression. However, Komine-Kobayashi et al40 showed that...
G-CSF significantly reduced iNOS levels and decreased the activation of iNOS-positive microglia after transient focal cerebral ischemia in mice. Current data suggest that additional studies are required to determine the effect of G-CSF on cytokine production after neurological insult and the importance of this pathway in G-CSF–induced neuroprotection.

**G-CSF Has an Antiapoptotic Effect on Neurons**

Apoptosis contributes to neuronal death in a variety of neurodegenerative diseases. Expanding evidence from recent studies clearly shows that G-CSF exerts an antiapoptotic effect on neurons both in vivo and in vitro. 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 cleavage. G-CSF exposure results in rapid STAT3 phosphorylation by JAK2 kinase in neurons, which is inhibited by AG 490, a specific inhibitor of JAK2 (Figure 2). G-CSF leads to an increase in the protein levels of the antiapoptotic STAT3 target Bcl-XL. Similarly, Schabitz et al showed that STAT3 expression in the penumbra of an infarction in a focal cerebral ischemia rat model was increased after G-CSF treatment. Komine-Kobayashi et al reported that the activation of the JAK2/STAT3 pathway by G-CSF activates Bcl-2 protein, which resulted in the inhibition of apoptotic cell death after transient focal cerebral ischemia in mice.

**Figure 2.** Signaling pathways suggested by the existing literature for G-CSF–mediated neuroprotection. G-CSFR activates the JAK/STAT, PI3K/PDK/Akt, and ERK family pathways. Activation of JAK2/STAT3 leads to increased expression of the antiapoptotic proteins bcl-xL and bcl-2 in neurons, which prevents apoptotic cell death by inhibiting activation of the caspases. Activation of the ERK family and PI3K/Akt also enhances neuronal survival.

It has been shown that G-CSF activates the PI3K/phosphoinositide-dependent kinase/Akt signaling pathway in cortical neurons in vitro. The antiapoptotic action of G-CSF on neurons has been suggested to be mediated at least partially by the PI3K/Akt pathway. It is well established that Akt, a downstream target of PI3K, is a critical antiapoptotic factor in controlling the balance between survival and apoptosis in multiple cell systems, including neurons, by several mechanisms, such as the phosphorylation of Bcl-2–associated death protein and caspase-9 and by inducing Bcl-2 expression. Phosphoinositide-dependent kinase has also been shown to phosphorylate and activate Akt to promote cell survival.

Finally, the measurement of the activation levels of the extracellular signal–regulated kinase (ERK) family showed that, whereas ERK1/2 was transiently and weakly activated by G-CSF, ERK5 kinase was strongly activated in cultured neurons from the rat cortex. ERK5, also known as big MAP kinase 1 (B MK1), is an MAP kinase member of which its biological role is largely undefined. However, it has been suggested that ERK5 activation enhances neuronal survival.

It is important to note that G-CSF exerts an antiapoptotic effect on many hematopoietic cell lines by triggering various signal pathways. For example, G-CSF inhibits spontaneous cytochrome C release and mitochondria-dependent apoptosis of myelodysplastic syndrome hematopoietic progenitors. However, additional studies are required to determine whether G-CSF maintains the viability of neuronal cells by similar mechanisms.

**G-CSF Drives Neurogenesis**

Neural stem cells (NSCs) are defined as undifferentiated cells that are capable of self-renewal, as well as being able to generate other cell types that constitute the CNS, including neurons, oligodendrocytes, and astrocytes. G-CSF has been shown to have a functional role in the differentiation of adult rat NSCs both in vitro and in vivo. In cultured NSC studies from the rat SVZ or hippocampus, analysis of the responsiveness of G-CSF has demonstrated that an increase in the cell number expressing mature neural markers, such as β III tubulin, neuron-specific enolase, and microtubule-associated protein-2, occurs. Peripheral infusion of G-CSF enhances the recruitment of progenitor cells from the lateral ventricular wall into the ischemic area of the neocortex in the rat. G-CSF increases hippocampal neurogenesis not only in ischemic animals but also in the intact, nonischemic rat. Based on this evidence, Schneider et al argued that G-CSF may enhance structural repair and function even in healthy subjects or may offer a novel therapeutic strategy for the treatment of chronic stroke patients.

Based on the available data, it can be hypothesized that G-CSF may play an important role in the augmentation of neurogenesis and neuroblast migration by activating the PI3K/Akt signaling pathway. As discussed earlier, the PI3K/Akt signaling pathway is an important target of G-CSF, which has also been shown to control neurogenesis. The PI3K/Akt pathway mediates cGMP-enhanced proliferation of adult progenitor cells derived from the SVZ of the rat. Furthermore, it has been suggested that the PI3K/Akt pathway also mediates neuroblast migration after stroke.
though both the activation of the PI3K/Akt signaling pathway in cortical neurons in vitro and the induction of neurogenesis by G-CSF have been shown previously, the relationships between these 2 complexes still remains to be elucidated.

The CXC chemokine receptor 4 (CXCR4)/stromal cell-derived factor-1 (SDF-1) system is essential for G-CSF–induced mobilization of hematopoietic progenitor cells into the peripheral blood. This system may also play an important role in G-CSF–induced neurogenesis. It has been shown that CXCR4 is expressed in rat and human neural progenitor cells and that the CXCR4/SDF-1 system is important in mediating specific migration of neural progenitor cells to the site of neurons damaged by ischemia. As discussed above, the neutralization of CXCR4 by its specific antibody has been reported to reduce the neuroprotective effect of G-CSF in rats after stroke. Although the ability of G-CSF to stimulate neurogenesis shows a lot of promise, additional studies are required to determine the molecular basis of this effect.

**Does G-CSF Mobilize Hematopoietic Stem Cells to the Injured Brain?**

It is well known that the administration of G-CSF mobilizes mainly CD34+ hematopoietic stem cells (HSCs) from the bone marrow into peripheral blood. The use of G-CSF has been shown to result in a significant decrease in infarct volumes and enhance survival rates, which has been suggested to be mediated by the mobilization of autologous HSCs after focal cerebral ischemia in mice. However, it is important to note that this study has no evidence to support the notion that the decrease in infarction volumes is through the mobilization of HSCs from bone marrow to the infarct region in the brain. Moreover, the precise mechanism and the role of HSC-mediated brain repair and/or neuroprotection after stroke is still controversial. Shyu et al showed that G-CSF treatment increases 5-bromodeoxyuridine–positive cells coexpressing the neuronal phenotypes of Neu-N and microtubule-associated protein-2 cells, as well as the glial phenotype of glial fibrillary acidic protein–positive cells in the ischemic cortical areas of G-CSF–treated rats after focal cerebral ischemia. They suggested that G-CSF treatment enhances translocation of HSCs into the ischemic brain and significantly improves lesion repair by improving neuronal plasticity and vascularization. Interestingly, the neutralization of CXCR4 has been associated with a slight reduction in infarction volume in G-CSF–treated rats. The CXCR4/SDF-1 system may control cerebral infiltration of CXCR4-carrying leukocytes during cerebral ischemia and contribute to ischemia-induced neuronal plasticity.

However, a more recent study by Komine-Kobayashi et al showed that bone marrow–derived cells are not involved in G-CSF–induced reduction of ischemic injury leading to contradictory results. They showed that G-CSF decreases the migration of ionized calcium binding adapter molecule–1/enhanced green fluorescent protein–positive bone marrow–derived monocytes/macrophages and increases intrinsic microglia/macrophages at the ischemic penumbra in a transient focal ischemia model of enhanced green fluorescent protein chimera mice. They concluded that the increased number of intrinsic microglia caused by G-CSF treatment may subsequently provide some neuroprotective effect. However, it should be noted that this neuroprotective role of microglial activation conflicts with other work showing that the inhibition of microglial activation results in neuroprotection.

**Other Neuroprotective Properties**

A recent study by Lee et al provides evidence that G-CSF treatment enhanced angiogenesis in a rat model of experimental stroke. They reported that G-CSF increased endothelial proliferation and vascular surface areas in the injured hemisphere and also increased endothelial NO synthase and angiopoietin-2 expression.
It is well established that excitotoxicity, especially glutamate, initiates a complex cascade of events that ultimately results in cell death in various types of CNS injury. The neuroprotective effect of G-CSF is also supported by the finding that G-CSF protects cerebellar granule cells exposed to glutamate in vitro.  

**Summary and Conclusions**

Although the underlying mechanisms of action remain to be elucidated, there are emerging data suggesting that G-CSF is a potential new agent for neuroprotection (Figure 3). The increased expression of G-CSF/G-CSFR on neurons subjected to hypoxia provides evidence that G-CSF may have an autocrine protective signaling mechanism in response to neural injury. G-CSF displays anti-inflammatory, antiapoptotic, and neurotrophic effects, all of which contribute to the neuroprotective properties of G-CSF. Moreover, G-CSF induces neurogenesis and angiogenesis after an ischemic insult. It produces neurogenesis and angiogenesis after an ischemic insult. Moreover, G-CSF in-  

**References**


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