

Granulocyte colony-stimulating factor potential use in the treatment of children with cerebral palsy

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ABSTRACT

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein that stimulates the bone marrow to produce granulocytes and stem cells and release them into the blood. Recent studies demonstrated the presence of CSF-receptor (G-CSFR) system in the brain and spinal cord, and their roles in neuroprotection and neural tissue repair, as well as improvement in functional

recovery. G-CSF exerts neuroprotective actions through the inhibition of apoptosis and inflammation, and the stimulation of neurogenesis. This review highlights recent studies on the potential use of G-CSF in cerebral palsy.

Keywords: Granulocyte colony-stimulating factor, neuroprotection, studies, cerebral palsy

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INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein that stimulates the bone marrow to produce granulocytes and stem cells and release them into the blood. Also, it induces the proliferation and differentiation of neutrophil precursors and mature neutrophils. G-CSF is widely used for treating and preventing chemotherapy-induced neutropenia. G-CSF is a cytokine and hormone as well. G-CSF is produced by the endothelium of blood vessels, macrophages, and a number of other immune cells. Moreover, G-CSF stimulates endothelial cells to proliferation and migration related to angiogenesis [1]. G-CSF is a strong inducer of hematopoietic stem cell mobilization from the bone marrow into the bloodstream [2].

Recent studies have demonstrated the presence of a CSF-receptor (G-CSFR) system in the brain and spinal cord, and their roles in neuroprotection and neural tissue repair as well as improvement in functional recovery. G-CSF receptors are expressed in pyramidal neurons in the cortex (mainly layers II/III and V), the Purkinje cell layer of the cerebellum, the hippocampus (hilus and CA3 field), the entorhinal cortex and the olfactory bulb. G-CSF is also expressed in the neurogenic regions in the adult brain: in the subgranular zone of the dentate gyrus and the subventricular zone [3].

Increased expression of G-CSF/G-CSFR in neurons subjected to hypoxia supports evidence that G-CSF has a protective signaling mechanism in response to neural damage. G-CSF exerts neuroprotective actions through the inhibition of apoptosis and inflammation, and the stimulation of neurogenesis. Furthermore, G-CSF has been shown to mobilize bone marrow stem cells into the injured brain, improving neural plasticity [4]. G-CSF also stimulates neurogenesis and synaptogenesis in the brain and prevents apoptosis. It has regenerative potential in various neurological disease models. [5,6].

Stem cells

Stem cells are undifferentiated cells that may specialize into multiple cell types and can self-renew. Stroke pathophysiology may be particularly amenable to stem cell therapy [7].

After the initial injury and associated changes, there is no enduring neurodegenerative process inhibiting recovery. The mechanism of stem cell therapy remains uncertain, and the three possible mechanisms include structural support for damaged or surrounding tissue re-myelination of the damaged axon, induction of regeneration via neurotrophic growth factor reduction TNF- α , IL- β , and IL-1 α level, and increase IL-6 level

The canonical niches for neural stem cells (NSCs) in the brain are the subventricular zone and

the dentate gyrus [7]. Changes in the migration patterns of neural progenitor cells (NPCs) following brain injury have been observed. Endogenous neural stem cells and cultured stem cells are capable of self-renewal and give rise to virtually all types of cells essential for the makeup of neuronal structures. Meanwhile, stem cells and neural progenitor cells are well-known for their potential for trophic support after transplantation into the ischemic brain [8,9].

Neurogenesis is stimulated by many factors such as: promoting pathways as potential methods to stimulate NPC proliferation, glial-derived neurotrophic factor, brain-derived neurotrophic factor, vascular endothelial growth factor, G-CSF, basic fibroblast growth factor-2, insulin-like growth factor-1, bone morphogenetic protein-7, epidermal growth factor, and transforming growth factor- α [10,11].

Experimental and clinical studies

Recent clinical studies investigated the dual role of G-CSF in the activation of bone marrow cells and neuroprotection. Ongoing clinical trials are investigating the dual roles of G-CSF, the activation of endogenous bone marrow cells, and neuroprotection to determine efficacy in stroke recovery. A recent review of 10 studies comprising 711 patients reported that G-CSF is safe and well tolerated. Moreover, G-CSF may foster functional recovery, as measured by the National Institutes of Health Stroke Scale and modified Rankin Scale scores [12].

Nishio et al. [13] evaluated the potential therapeutic effect of G-CSF for spinal cord injury in mice. They found that G-CSF is neuroprotective against glutamate-induced cell death of cerebellar granule neurons *in vitro*. Also, histologic examination revealed that the number of surviving neurons in the spinal cord was increased. An immunohistochemistry study confirmed that G-CSF suppressed neuronal apoptosis after SCI. Moreover, administration of G-CSF promoted hindlimb functional recovery.

G-CSF in a mouse spinal cord hemisection recruits microglia to the injury site in the first 72h after spinal cord injury [14]. Also, G-CSF reduces the expression of pro-inflammatory factors and activates the expression of neurotrophic factors. Furthermore, G-CSF induces the expression of markers of M2 macrophage and inhibits the expression of markers of M1 macrophage in BV2 microglia in an *in vitro* model. The authors suggested that the administration of G-CSF within the first 72h after spinal cord injury might reduce early inflammation-induced detrimental effects and promote an anti-inflammatory response that favors repair via improving alternative activation of microglia.

In an experimental study of neonatal hypoxia-ischemia, G-CSF+ stem cell factor (SCF) coadministration showed significant improvement in neurological function. The administration of G-CSF in combination with SCF not only prevented brain atrophy but also improved neurological function [15].

In a recent experimental study of a neonatal animal model of excitotoxic brain injury, [16] showed that G-CSF protects against N-methyl-D-aspartate receptor-mediated developmental excitotoxic brain damage. The neuroprotective effects in this model of excitotoxic brain injury depended on the timing of drug administration after the insult.

Also, Lu et al. [17] investigated the potential protective effects of G-CSF in a gerbil model of global cerebral ischemia. They examined neuronal death, inflammatory reaction, and neurogenesis in the hippocampus 72 h after transient forebrain ischemia and functional deficits. G-CSF at 25-50 $\mu\text{g}/\text{kg}$ significantly reduced neuronal loss in the hippocampus CA1 area, but not at 10 $\mu\text{g}/\text{kg}$. G-CSF at 50 $\mu\text{g}/\text{kg}$ reduced the level of TNF- α . Furthermore, the number of cells in the hippocampal dentate gyrus increased with G-CSF treatment. The authors concluded that the neuroprotective effects of G-CSF may be related to a reduction of inflammation and increased neurogenesis in the hippocampus.

Rah et al. [17], in a randomized, double-blind study, evaluated the neuroregenerative potential of intravenous G-CSF followed by infusion of mobilized peripheral blood mononuclear cells (mPBMCs) in 57 children with cerebral palsy. Patients who received G-CSF followed by mPBMC infusion at 7 months (T7 group) demonstrated significantly more neurodevelopmental improvement than patients who received G-CSF followed by mPBMC infusion at 1 month (T1 group). They also found metabolic changes to the cerebellum, thalamus, and cerebral cortex in the 18F-FDG brain PET-CT scans. No significant differences in such changes between the mPBMC and placebo group or between the T1 and T7 group were found.

Recent studies revealed that persistent neuroinflammation and associated apoptosis in brains affected by CP could be therapeutic targets. Apoptosis is an attractive target because anti-apoptotic agents could be used to reverse apoptosis during a therapeutic time window after hypoxia-induced injury [18]. Other potential therapeutic targets include hematopoietic growth factors, such

as erythropoietin (EPO) and G-CSF, which influence the proliferation of neural stem and progenitor cells. EPO and G-CSF have specific receptors in the brain and both factors are produced in the brain [Brines, 2005; Schneider, 2005]. Therefore, EPO and G-CSF have been investigated for their ability to stop neurodegenerative conditions [19,20].

Hoh et al. [21] investigated the therapeutic mechanism of cell therapy in patients with cerebral palsy using G-CSF mobilized mPBMCs. They compared the expression of inflammatory cytokines and neurotrophic factors in PBMCs and mPBMCs from children with cerebral palsy to those from healthy adult donors and to cord blood mononuclear cells donated from healthy newborns. In cerebral palsy children, the expression of interleukin-6 was significantly increased in mPBMCs compared with PBMCs. The expression of brain-derived neurotrophic factors in mPBMC from children with cerebral palsy was significantly higher than that in cord blood or mPBMCs from healthy adults. The authors suggested that mPBMCs have the potential to become seed cells for the treatment of cerebral palsy.

Park et al. [22] examined intravenous infusion of peripheral blood mononuclear cells (mPBMC) mobilized by G-CSF on upper extremity function in 47 children with cerebral palsy. G-CSF was given for 5 days, mPBMC was collected and cryopreserved. Twenty-two patients received mPBMC and 25 patients received placebo. Six months later, the patients were switched, and administered mPBMC and placebo, respectively. The Quality of Upper Extremity Skills Test (QUEST) and the Manual Ability Classification System (MACS) were used to assess upper motor function. Total QUEST scores were significantly improved after mPBMC and placebo infusion. The level of MACS did not change in both groups. The authors suggested that the effect of mPBMC was masked by the influence of G-CSF, which has neurotrophic potentials in children with cerebral palsy.

There are many examples of the importance of G-CSF in brain and neurological diseases [6,12,17,18,22,23]. We hope, that this review highlights the potential role of G-CSF in the treatment of cerebral palsy

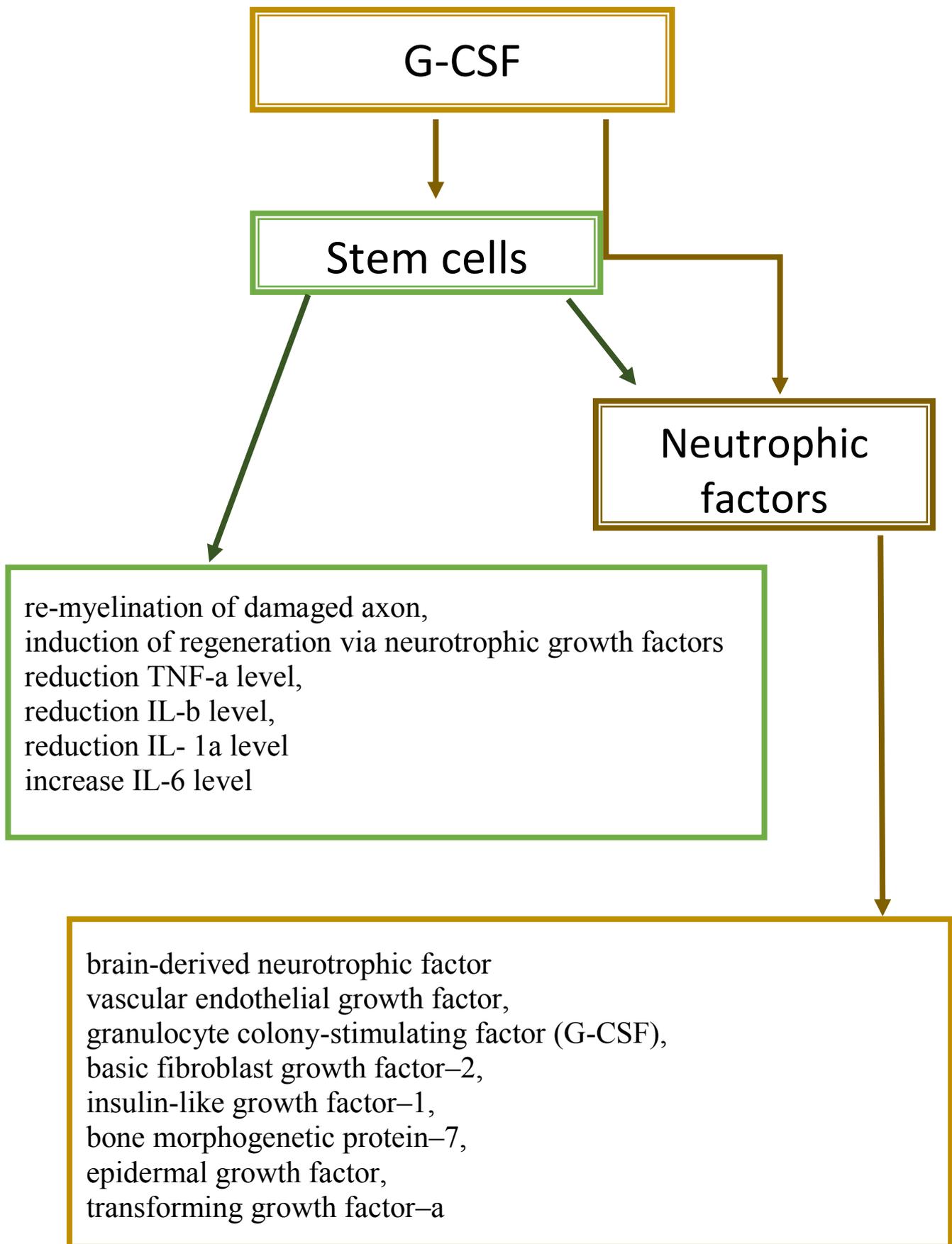


Figure 1. Effects of granulocyte colony-stimulating factor (G-CSF) on stem cells and stimulation neurotrophic factors

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

1. Bussolino F, Ziche M, Wang JM, Alessi D, Morbidelli L, Cremona O, Bosia A, Marchisio PC, Mantovani A. In vitro and in vivo activation of endothelial cells by colony-stimulating factors. *J Clin Invest.* 1991 Mar;87(3):986-95
2. Thomas J, Liu F, Link DC. Mechanisms of mobilization of hematopoietic progenitors with granulocyte colony-stimulating factor. *Curr Opin Hematol.* 2002 May;9(3):183-9.
3. Schneider A, Kuhn HG, Schäbitz WR. A role for G-CSF (granulocyte colony stimulating factor) in the central nervous system. *Cell Cycle.* 2005;4:1753-7.
4. Solaroglu I, Jadhav V, Zhang JH. Neuroprotective effect of granulocyte-colony stimulating factor. *Front Biosci.* 2007 Jan 1;12:712-24.
5. Pitzer C, Klusmann S, Krüger C, Letellier E, Plaas C, Dittgen T, Kirsch F, Stieltjes B, Weber D, Laage R, Martin-Villalba A, Schneider A. The hematopoietic factor granulocyte-colony stimulating factor improves outcome in experimental spinal cord injury. *J Neurochem.* 2010 May;113(4):930-42.
6. Kawabe J, Koda M, Hashimoto M, Fujiyoshi T, Furuya T, Endo T, Okawa A, Yamazaki M. Neuroprotective effects of granulocyte colony-stimulating factor and relationship to promotion of angiogenesis after spinal cord injury in rats: laboratory investigation. *J Neurosurg Spine.* 2011 Oct;15(4):414-21.
7. Wei L, Wei ZZ, Jiang MQ, Mohamad O, Yu SP. Stem cell transplantation therapy for multifaceted therapeutic benefits after stroke. *Prog Neurobiol.* 2017 Mar 18.
8. Anenberg E, Arstikaitis P, Niitsu Y, Harrison TC, Boyd JD, Hilton BJ, Tetzlaff W, Murphy TH. Ministrokes in channelrhodopsin-2 transgenic mice reveal widespread deficits in motor output despite maintenance of cortical neuronal excitability. *J Neurosci.* 2014 Jan 22;34(4):1094-104.
9. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med.* 2002; Sep;8(9):963-70.
10. Dempsey RJ, Sailor KA, Bowen KK, Türeyen K, Vemuganti R. Stroke-induced progenitor cell proliferation in adult spontaneously hypertensive rat brain: effect of exogenous IGF-1 and GDNF. *J Neurochem.* 2003;87:586-97.
11. Peña Id, Borlongan CV. Translating G-CSF as an Adjunct Therapy to Stem Cell Transplantation for Stroke. *Transl Stroke Res.* 2015 Dec;6(6):421-9.
12. Kawada H, Takizawa S, Takanashi T, Morita Y, Fujita J, Fukuda K, Takagi S, Okano H, Ando K, Hotta T. Administration of hematopoietic cytokines in the subacute phase after cerebral infarction is effective for functional recovery facilitating proliferation of intrinsic neural stem/progenitor cells and transition of bone marrow-derived neuronal cells. *Circulation.* 2006 Feb 7;113(5):701-10.
13. Nishio Y, Koda M, Kamada T, Someya Y, Kadota R, Mannoji C, Miyashita T, Okada S, Okawa A, Moriya H, Yamazaki M. Granulocyte colony-stimulating factor attenuates neuronal death and promotes functional recovery after spinal cord injury in mice. *J Neuropathol Exp Neurol.* 2007 Aug;66(8):724-31.
14. Guo Y, Zhang H, Yang J, Liu S, Bing L, Gao J, Hao A. Granulocyte colony-stimulating factor improves alternative activation of microglia under microenvironment of spinal cord injury. *Neuroscience.* 2013 May 15;238:1-10.
15. Doycheva D, Shih G, Chen H, Applegate R, Zhang JH, Tang J. Granulocyte-colony stimulating factor in combination with stem cell factor confers greater neuroprotection after hypoxic-ischemic brain damage in the neonatal rats than a solitary treatment. *Transl Stroke Res.* 2013 Apr;4(2):171-8.
16. Neubauer V, Wegleiter K, Posod A, Urbanek M, Wechselberger K, Kiechl-Kohlendorfer U, Keller M, Griesmaier E. Delayed application of the haematopoietic growth factors G-CSF/SCF and FL reduces neonatal excitotoxic brain injury. *Brain Res.* 2016 Mar 1;1634:94-103.
17. Rah WJ, Lee YH, Moon JH, Jun HJ, Kang HR, Koh H, Eom HJ, Lee JY, Lee YJ, Kim JY, Choi YY, Park K, Kim MJ, Kim SH. Neuroregenerative potential of intravenous G-CSF and autologous peripheral blood stem cells in children with cerebral palsy: a randomized, double-blind, cross-over study. *J Transl Med.* 2017 Jan 21;15(1):16.
18. Goni-de-Cerio F, Alvarez A, Caballero A, Mielgo VE, Alvarez FJ, Rey-Santano MC, et al. Early cell death in the brain of fetal preterm lambs after hypoxic-ischemic injury. *Brain Res.* 2007;1151:161-71.
19. Wang H, Zhang L, Jin Y. A meta-analysis of the protective effect of recombinant human erythropoietin (rhEPO) for neurodevelopment in preterm infants. *Cell Biochem Biophys.* 2015;71(2):795-802.
20. Grassinger J, Khomenko A, Hart C, Baldaranov D, Johannesen SW, Mueller G. Safety and feasibility of long term administration of recombinant human granulocyte-colony

- stimulating factor in patients with amyotrophic lateral sclerosis. *Cytokine*. 2014;67(1):21–8.
21. Hoh H, Hwang K, Lim HY, Kim YJ, Lee YH. Mononuclear cells from the cord blood and granulocyte colony stimulating factor-mobilized peripheral blood: is there a potential for treatment of cerebral palsy? *Neural Regen Res*. 2015 Dec;10(12):2018-24.
 22. Park KI, Lee YH, Rah WJ, Jo SH, Park SB, Han SH, Koh H, Suh JY, Um JS, Choi EH, Park UJ, Kim MJ. Effect of Intravenous Infusion of G-CSF-Mobilized Peripheral Blood Mononuclear Cells on Upper Extremity Function in Cerebral Palsy Children. *Ann Rehabil Med*. 2017 Feb;41(1):113-20.
 23. Sairanen M, Lucas G, Ernfors P, Castrén M, Castrén E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci*. 2005; 25:1089–94.