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The Role of Stem Cell Factor and Granulocyte-Colony Stimulating Factor in Treatment of Stroke

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Abstract

Stroke is a serious cerebrovascular disease that causes high mortality and persistent disability in adults worldwide. Stroke is also an enormous public health problem and a heavy public financial burden in the United States. Treatment for stroke is very limited. Thrombolytic therapy by tissue plasminogen activator (tPA) is the only approved treatment for acute stroke, and no effective treatment is available for chronic stroke. Developing new therapeutic strategies, therefore, is a critical need for stroke treatment. This article summarizes the discovery of new routes of treatment for acute and chronic stroke using two hematopoietic growth factors, stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF). In a study of acute stroke, SCF and G-CSF alone or in combination displays neuroprotective effects in an animal model of stroke. SCF appears to be the optimal treatment for acute stroke as the functional outcome is superior to G-CSF alone or in combination (SCF+G-CSF); however, SCF+G-CSF does show better functional recovery than G-CSF. In a chronic stroke study, the therapeutic effects of SCF and G-CSF alone or in combination appear differently as compared with their effects on the acute stroke. SCF+G-CSF induces stable and long-lasting functional improvement; SCF alone also improves functional outcome but its effectiveness is less than SCF+G-CSF, whereas G-CSF shows no therapeutic effects. Although the mechanism by which SCF+G-CSF repairs the brain in chronic stroke remains poorly understood, our recent findings suggest that the SCF+G-CSF-induced functional improvement in chronic stroke is associated with a contribution to increasing angiogenesis and neurogenesis through bone marrow-derived cells and the direct effects on stimulating neurons to form new neuronal networks. These findings would assist in developing new treatment for stroke. The article presents some promising patents on role of stem cell factor and granulocyte-colony stimulating factor in treatment of stroke.

Keywords

Acute stroke; chronic stroke; G-CSF; hematopoietic growth factors; neuroprotection; neuronal plasticity; SCF

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

INTRODUCTION

A stroke is caused by a sudden disruption of the blood flow to a brain region. In the United States, stroke is the fourth leading cause of death and a leading cause of serious and long-term disability in adults. Stroke is not only a serious neurological disorder but also represents a heavy public financial burden in the United States. The annual healthcare cost for stroke in 2008 was \$34.3 billion [1]. Based on the pathological progression and the timing after stroke onset, a stroke is classified into three phases: acute stroke, subacute stroke and chronic stroke. Although the duration of the three phases of stroke is dependent on the location and the size of brain infarction (dead brain tissue) and the age of patients, in general, acute stroke is described as the first 48 h after stroke onset, subacute stroke is 48 h to 6 weeks or to 3 months post-stroke, whereas chronic stroke is beyond 3 to 6 months after stroke onset [2–4].

The treatment for stroke is limited. Administering recombinant tissue plasminogen activator (rtPA) within 4.5 h of stroke onset for thrombolysis is the only approved drug for treatment of ischemic stroke patients in the acute phase [5, 6]. Because of the narrow therapeutic window of rtPA and rtPA-induced intracerebral hemorrhage, most stroke patients are not able to receive this thrombolytic therapy. If they survive acute and subacute stroke, they become chronic stroke patients. Currently, no effective treatment is available for chronic stroke. Therefore, developing new therapeutic strategies is a critical need to improve the health of stroke patients.

Stroke is a unique neurological disorder starting with sudden brain tissue death (brain infarction) and then followed by brain repair and recovery. Truly understanding the pathological features and unique progression of this specific disorder is the key to find the cure of stroke. Recently, Moskowitz and colleagues have emphasized the involvement of neurons, glia, vascular cells and matrix components as a neurovascular unit in brain tissue death and brain repair in the setting of stroke [7]. Cell-cell interaction and communication among all cell types in the brain including infiltrated blood-borne cells, and a wide variety of molecules that are overproduced by ischemic cells might participate in the process of brain tissue death during the acute phase of stroke. Therefore, the therapeutic approaches targeting only on a single molecule, a single cell type or a single event might be not sufficient to rescue the entire neurovascular unit from ischemic injury. This notion is supported by the evidence that the use of NMDA and AMPA blocker, nitroxide-based radical spin trap, or inflammatory inhibitor to protect brain damage from stroke has failed in clinical trials [8–10]. On the other hand, the pathological features of acute stroke, subacute stroke and chronic stroke, and the mechanisms behind neurovascular-unit injury and repair are quite different; therefore, the therapeutic strategies for each setting should be specific and cooperative. The challenging, however, is that the precise boundary between the timing of neurovascular-unit injury and repair is difficult to be distinguished. It is often seen that some targeting molecules, such as NMDA receptor, matrix metalloproteinases and the intracellular mediator HMGB1, may have neuroprotective benefits in the acute phase of stroke but they may also risk negatively influencing brain repair in the chronic phase or recovery phase [7]. It has been documented that adenosine, a nucleoside, is extensively produced in the brain under the condition of ischemia, and that adenosine protects neurons through inhibiting excitatory synaptic neurotransmission in the ischemic brain [11]. Although many studies have revealed the neuroprotective effects of adenosine in animal models of stroke [11–13], some other findings display no neuroprotective effects or even show detrimental effects [12–14]. It has not been elucidated whether adenosine-induced inhibitory effect on neuronal activity [15] and blockage of glutamate release [11] will negatively affect neuronal network remodeling during the recovery phase as glutamate is involved in brain recovery after ischemic injury [16]. Thus, ideal candidates for the

treatment of stroke should have the effects in both neuroprotection and neural network rewiring.

Hematopoietic stem cells or hematopoietic progenitor cells (HSCs/HPCs) are the bone marrow cells that generate blood cells. The survival, proliferation and differentiation of HSCs/HPCs are controlled by hematopoietic growth factors. Stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) are the essential members of the hematopoietic growth factor family. SCF and G-CSF were originally discovered as hematopoietic growth factors two decades ago based on their effectiveness in supporting HSC/HPC survival and growth [17, 18]. SCF and G-CSF are produced by bone marrow stromal cells and fibroblasts [19, 20], whereas receptors for SCF (ckit) and G-CSF (G-CSFR) are expressed on HSCs/HPCs [21, 22]. It has been demonstrated that SCF and G-CSF are critically involved in the regulation of HSC/HPC proliferation, differentiation and mobilization [23, 24]. Research data collected from both patients and laboratory animals including rodents, canines and nonhuman primates have supported that SCF in combination with G-CSF (SCF+G-CSF) has synergistic effects on the mobilization of HSCs/HPCs from the bone marrow to the blood stream [25].

In addition to the effects of SCF and G-CSF on hematopoietic system, recent studies have suggested that SCF and G-CSF may also play roles in the central nervous system. Many studies have demonstrated that administration of SCF and G-CSF in ischemic stroke has beneficial effects [26]. It has been shown that systemic administration of SCF [27] and G-CSF alone [27–32] or SCF in combination with G-CSF [27, 33] during the acute phase of experimental stroke reduces infarct size and ameliorates brain ischemia-induced neurological deficits. Sevimli and co-workers [34] reported that endogenous G-CSF is neuroprotective in the setting of experimental stroke. Both SCF [35] and G-CSF [31, 35] have been shown to pass through the blood-brain barrier (BBB) in intact rats. In addition, SCF+G-CSF treatment during 10–20 days after focal brain ischemia also displays beneficial effects on reduction in infarct size and improvement of functional outcome in a mouse model of ischemic stroke [36]. Furthermore, systemic administration of SCF+G-CSF beginning at 3.5 months after induction of experimental stroke improves functional recovery, increases neuronal activity in the lesioned hemisphere [37], enhance angiogenesis and neurogenesis [38], and promotes neural network remodeling in the peri-infarct cortex (Cui *et al.*, unpublished observation). Taken together, these findings suggest that SCF+G-CSF treatment has a much wider therapeutic window for ischemic stroke and that this treatment provides the beneficial effects on both brain protection and brain repair. Therefore, SCF and G-CSF combination therapy would be a good candidate for a new therapy for stroke. A summary of recent findings concerning the therapeutic effects of SCF and G-CSF on brain ischemia was listed in Table 1. This article, based on our recent findings and patents, highlights the discovery of the therapeutic effects of SCF and G-CSF in the treatment of acute and chronic stroke.

RECEPTORS FOR SCF AND G-CSF ARE EXPRESSED ON NEURONS IN THE ADULT BRAIN

Before determining the therapeutic effects of SCF and G-CSF on brain ischemia, we first examined whether the receptors for SCF and G-CSF are expressed on neurons in the brain. Through immunohistochemistry we observed that both ckit and GCSFR, the receptors for SCF and G-CSF respectively, were expressed in the neurons of adult rat brains [27]. Fig. (1) shows that rat cortical neurons in adult brain carry the SCF receptor, ckit, and the G-CSF receptor, GCSFR. Similar findings have also been observed by other investigators. Ckit was found to express in the cultured neurons [39], and GCSFR was expressed in the cortical neurons in the brain [28]. These findings suggest that the hematopoietic growth factors, SCF

and G-CSF, may have biological function on the cortical neurons. This biological function may include but is not limited to neuroprotection and/or neuronal plasticity.

NEUROPROTECTIVE EFFECTS OF SCF AND G-CSF IN ACUTE STROKE

We then examined whether SCF and G-CSF have neuroprotective effects in acute stroke. SCF and G-CSF alone or in combination was administered subcutaneously for 7 days, beginning at 3 h after induction of cortical brain ischemia in spontaneously hypertensive rats (SHRs). Male adult SHRs were used for this study. The SHRs were subjected to the cortical brain ischemia by permanent ligation of the right common carotid artery (CCA) and middle cerebral artery (MCA). The similar procedures were performed in sham-operative SHRs except the ligation of both CCA and MCA [27]. Functional recovery was evaluated with limb placement and foot-fault tests 1, 4, 7 and 10 weeks after treatment. The limb placement test was used to examine the somatosensory and motor function for the four limbs. Animals were examined at 8 different conditions. Each side of limbs was scored between 0–16. Score 0 stands for severe neurological deficits, whereas score 16 represents no neurological deficits. In foot-fault test, the hindlimbs were gently held up while rats were walking across a wire grid, and the number of slippages of the affected forepaws between grids was recorded. The detailed methods for both limb placement and foot-fault tests have been described elsewhere [40, 41]. Infarction size was determined using an indirect measurement method [40] through serial coronal brain sections at 10 weeks post-ischemia. We found that SCF and G-CSF alone or in combination treatment significantly improved functional outcomes Fig. (2A & B) and reduced the infarction size Fig. (2C) [27]. Interestingly, different degree of functional improvement was also observed among SCF, G-CSF, and SCF +G-CSF groups. At both limb placement and foot-fault tests SCF alone treatment showed the best functional outcome among the treatment groups because cortical infarct-induced impairment of somatosensorimotor function was improved as early as 1 week after SCF treatment, and SCF-induced functional improvement was also detected 4, 7 and 10 weeks after treatment. SCF+G-CSF treatment also showed a long-lasting functional improvement during 4–10 weeks after treatment whereas SCF+G-CSF-induced improvement was not seen 1 week post-treatment according to the animals' performance on both behavioral tests. G-CSF alone only induced functional recovery at 1 and 10 weeks post-treatment, but it did not show improvement 4 or 7 weeks after treatment in the limb placement test. In the foot-fault test, G-CSF did not show significant functional improvement [27] Fig. (2A & B). Based on the behavioral scores of limb placement test, somatosensorimotor function was restored about 66%, 41%, 40%, or 23% in the groups of SCF, G-CSF, SCF+G-CSF, or saline control respectively at 1 week post-treatment, and the SCF-induced functional recovery was significantly greater than other 3 groups. In addition, about 97%, 93%, 53% or 36% functional restoration was seen in SCF+G-CSF, SCF, G-CSF, or saline controls respectively at 10 weeks post-treatment. The SCF+G-CSF- and SCF-induced functional recovery was significantly greater than G-CSF alone and saline controls, and G-CSF caused a significant recovery as compared to saline controls 10 weeks after treatment. In addition to the superior functional outcome, SCF alone treatment also showed a trend toward decreased infarction size as compared to G-CSF or SCF+G-CSF. Taken together, these findings suggest that SCF and G-CSF alone or in combination has neuroprotective effects on acute stroke and that SCF is more effective than G-CSF in the treatment of acute stroke.

It remains uncertain why and how SCF provides better therapeutic effects on acute stroke than G-CSF alone in SHRs. It is worth noting that SCF treatment for acute stroke in SHRs causes a significant increase in neural stem cell proliferation in the neurogenic regions whereas G-CSF alone did not show the similar effect [27]. A recent study revealed that brain ischemia-induced neural stem cell proliferation in the neurogenic regions is required for neuron protection in acute stroke [42]. In addition, G-CSF alone treatment led to a

significant reduction in body weight 1 week after the treatment [27]. G-CSF-induced body weight loss may have a negative influence on functional recovery.

THE EFFECTS OF SCF AND G-CSF ON BRAIN REPAIR IN CHRONIC STROKE

Next we determined the efficiency of SCF and G-CSF in neuronal plasticity in an animal model of chronic stroke. Chronic stroke is defined as the time beyond 3–6 months after stroke onset. Unlike in acute and subacute stroke, when the brain is suffering from primary and secondary neuron death, neuron loss is not a major problem in chronic stroke [2–4]. Surviving neurons in the nonlesioned brain regions take over the function of the lost neurons in the infarct zone [43] during the chronic phase; therefore, chronic stroke is considered the recovery stage of stroke. Currently, no effective treatment is available for chronic stroke patients other than physiotherapy. A therapeutic intervention that can enhance neuronal plasticity and rebuild neuronal networks may provide a new treatment for chronic stroke.

Do SCF and G-CSF have potential therapeutic effects on chronic stroke? Before addressing this question, one thing that we must consider is to determine whether SCF and G-CSF can pass through the BBB because the BBB is reestablished in the chronic stroke phase. To examine the BBB permeability to SCF and G-CSF, I¹²⁵-labeled SCF or G-CSF was intravenously injected to the adult rats that did not subject to brain ischemia, and both blood samples and brain samples were collected at 10, 30, 60, or 120 min after injection. We observed that SCF and G-CSF were slowly but continuously transported from the blood into the brain, suggesting that SCF and G-CSF can pass through the BBB in the physiological condition (intact condition) [35]. In addition, receptors for SCF and G-CSF were also found to express on cerebral endothelial cells, suggesting that SCF and G-CSF may cross the BBB through a receptor-mediated transport system [35].

Next we sought to determine the therapeutic effects of SCF and G-CSF in chronic stroke. SCF and G-CSF alone or in combination were subcutaneously administered for 7 days beginning at 3.5 months after the induction of cortical brain ischemia in SHRs. Functional recovery was evaluated with limb placement and foot-fault tests 1, 5, and 17 weeks after the final injection Fig. (3A). We observed that the best functional outcome was seen in the SCF +G-CSF-treated group because SCF+G-CSF-treated rats received the highest scores in the limb placement test and showed lowest foot faults in the foot-fault test as compared to the treatment controls, SCF or G-CSF alone treatment at 1, 5, and 17 weeks post-treatment, suggesting a stable and a long-lasting functional improvement by SCF+G-CSF treatment Fig. (3B & C) [37]. SCF alone treatment also displayed an improved functional outcome, but SCF-induced recovery was not as stable as SCF+G-CSF Fig. (3B & C) [37]. G-CSF-treated rats did not show any functional improvement Fig. (3B & C) [37]. In addition, when analyzing the degree of functional restoration, we found that at 17 weeks after treatment somatosensorimotor function was restored about 88%, 69%, 50%, or 44% in SCF+G-CSF, SCF, G-CSF, or saline controls according to the behavioral scores. The SCF+G-CSF-induced recovery was significantly greater than G-CSF and saline controls, and SCF also significantly improved recovery when compared to G-CSF and saline controls. To further confirm the behavioral examination data, neuronal activity in the somatosensory cortex of both hemispheres was recorded by field-evoked potential when electronically stimulating the forepaws 2 weeks after the final behavioral test. Interestingly, the electrophysiological data showed the similar pattern of the behavioral test data. We found that only SCF+G-CSF treatment showed a significant increase in neuronal activity in the lesion side brain (right brain) as compared to the unlesioned brain when stimulating the affected forepaw (left side), suggesting that a normal somatosensory pathway may be reestablished between the affected forepaw and the lesion side brain by SCF+G-CSF. Although a similar pattern of field-

evoked potential was observed in SCF alone treatment, the SCF-induced increase in neuronal activity in the lesion side brain did not reach a significant level as compared to the contralateral unlesioned brain. G-CSF alone did not cause significant increase in neuronal activity in the right lesioned brain when stimulating the left affected forepaw Fig. (4) [37]. Together, these chronic stroke studies indicate that SCF+G-CSF and SCF but not G-CSF alone treatment in chronic stroke induces functional restoration, and that SCF+G-CSF intervention appears to be an optimal paradigm for brain repair in the setting of chronic stroke.

The research work stated above has been included into the patents: Zhao LR, Kessler J, Singhal S, and Mehta J. Use of SCF and G-CSF in the treatment of cerebral ischemia and neurological disorders. WO2006055260 (2006), CA2586365 (2006), AU-B-2005306894 (2011), and US20120286039A1 (2012) (see detailed information in Table 2). Other patents that are related to the use of SCF and/or G-CSF for the treatment of brain ischemia were also listed in Table 2.

CURRENT AND FUTURE DEVELOPMENTS

Although our studies have demonstrated the therapeutic effects of SCF and G-CSF on both acute and chronic stroke, the mechanisms in which SCF and G-CSF protect or repair the brain in stroke remains poorly understood.

Recently, we have determined the contribution of SCF+G-CSF to brain repair in chronic stroke. Our findings, which will be presented later in this section, suggest that repair of the brain in chronic stroke by SCF+G-CSF is manipulated through an indirect effect on bone marrow-derived cells and a direct effect on cortical neurons.

To track bone marrow-derived cells, the bone marrow of stroke animals was replaced with the bone marrow of transgenic mice carrying a green fluorescent protein (GFP). Male adult C57BL mice were subjected to cortical brain ischemia by permanent occlusion of the right CCA and MCA [38]. The stroke mice were received a lethal dose of x-ray irradiation to destroy the bone marrow, thereafter, the bone marrow of GFP mice (10^7 cells/mouse) was transplanted into the irradiated mice via the tail vein within 24 h after the irradiation. One month after bone marrow transplantation, SCF+G-CSF was subcutaneously injected for 7 days initiating at 3.5 months after induction of cortical brain ischemia. We found that SCF+G-CSF treatment in chronic stroke significantly elevated bone marrow stem cells in the blood, suggesting the efficiency of SCF+G-CSF on bone marrow stem cell mobilization in the setting of chronic stroke [38]. Furthermore, bone marrow-derived endothelial cells (CD31⁺/GFP⁺ cells) in the brain were significantly increased by SCF+G-CSF Fig. (5A–C) [38]. Moreover, blood vessel density in the peri-infarct cortex was also increased in the brains of SCF+G-CSF-treated mice Fig. (5D–F) [38], indicating an angiogenic effect of SCF+G-CSF in chronic stroke. In addition to the enhancement of angiogenesis, SCF+G-CSF treatment in chronic stroke also augmented bone marrow-derived neurons (NeuN⁺/GFP⁺ cells) in the cortex bordering the infarct cavity Fig. (5G–I) [38]. These data suggest that SCF+G-CSF may repair the brain of chronic stroke through the enhancement of the differentiation of bone marrow stem cells into cerebral endothelial cells and cortical neurons. Whether the enhancement of angiogenesis is required for SCF+G-CSF-induced functional improvement in chronic stroke and whether bone marrow-derived neuronal like cells can form functional synaptic networks with existing neuronal circuits in the peri-infarct cortex will be determined in the future studies.

In addition to the involvement of bone marrow-derived cells in brain repair by SCF+G-CSF, SCF+G-CSF can also enhance neuronal network remodeling in the peri-infarct cortex in chronic stroke. Dendritic spines are the postsynaptic membranes that form synaptic

connections with the axonal terminals of excitatory neurons [44]. Thus, dendritic spine density can serve as an index for determination of neuronal network formation. Of the dendritic spines, only mushroom-type spines can build up stable and functioning synaptic networks because the mushroom-type spine has a large head supported by endogenous organelles, such as smooth endoplasmic reticulum [45], endosomal compartments [46], polyribosomes [47] and perisynaptic astroglia [48], and the mushroom-type spine also contains a high density of glutamate receptors [49]. When examining dendritic spines in the pyramidal neurons surrounding the infarct cavities of chronic stroke brains, we found that SCF+G-CSF induced a 64% increase in mushroom-type spines Fig. (6C). Moreover, dendritic branching in the peri-infarct cortex was also enhanced by SCF+G-CSF Fig. (6D–F). These data suggest that SCF+G-CSF treatment in chronic stroke enhances the establishment of stable and functioning neuronal networks in the cortex adjacent to the infarct cavities. The SCF+G-CSF-induced neuronal network remodeling may facilitate and enhance the capability of surviving neurons to take over the function of dead neurons in the infarct region. These results would help us understand why SCF+G-CSF induces a stable and long-lasting functional improvement when administered in the chronic stroke phase. To further determine whether SCF+G-CSF can directly stimulate neurons to form neuronal networks, we conducted *in vitro* studies using primary cortical neuronal cultures. We observed that neurite outgrowth and neuronal network formation were significantly enhanced by SCF+G-CSF Fig. (6G–I). In addition, we also revealed that PI3/AKT signaling, NFkB and brain-derived neurotrophic factor were critically involved in SCF+G-CSF-induced neurite outgrowth (Su *et al.*, unpublished observation).

Most interestingly, using a live brain imaging approach, our recent work carried with aged stroke mice has revealed that SCF+G-CSF treatment results in an increase in mushroom spines in the peri-infarct cortex and a decrease in brain infarction-induced synaptic loss in the cortical neurons adjacent to the infarct cavities. This observation suggests that an aged brain with chronic stroke can be also repaired by SCF+G-CSF treatment through the enhancement of new neuronal network formation (Cui *et al.*, unpublished observation). This work is important because the aged population has a high incidence of stroke. It would also help in developing new therapeutic strategies to treat aging-related neurological disorders or neurodegenerative diseases.

It is to be expected that this research concerning the therapeutic role of SCF and G-CSF in stroke can be easily translated into clinical trials as SCF+G-CSF has been proven safe and effective in other medical purpose for mobilizing bone marrow stem cells [50–60].

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Fig. 1.

Expression of SCF and G-CSF receptors in cortical neurons of adult intact brain. Brain sections were processed for immunofluorescent staining. Ckit (red in A and B), receptor for SCF. GCSFR (red in C), receptor for G-CSF. Green: NeuN, the neuronal marker. DAPI: nuclear counterstain. Bar=20 μ m: bar in A, indicator for A and B; bar in F, indicator for C–F. Note that cortical neurons show positive immunoreaction for ckit and GCSFR. Reproduced from Zhao LR, Singhal S, Duan WM, Mehta J, Kessler JA. Brain repair by hematopoietic growth factors in a rat model of stroke. *Stroke*. 2007; 38:2584–2591, with permission of publisher.

Fig. 2.

Neuroprotection by SCF and G-CSF in acute stroke. **A:** Functional outcome examination by limb placement test. Note that no neurological deficits are found before induction of stroke (MCAO: middle cerebral artery occlusion) in spontaneously hypertensive rats, however, severe neurologic deficits are seen in all stroke rats at 3 h post-MCAO as compared to sham rats (* $p < 0.01$). SCF and G-CSF alone or in combination all induce functional improvement when compared to saline treatment controls 1–10 weeks after treatment. Week 1: * $p < 0.01$, SCF vs. saline; † $p < 0.05$, SCF vs. G-CSF and SCF+G-CSF, or G-CSF vs. saline. Week 4: * $p < 0.01$, SCF vs. saline, SCF+G-CSF vs. saline or G-CSF. † $p < 0.05$, SCF vs. G-CSF. Week 7: * $p < 0.01$, SCF or SCF+G-CSF vs. saline or G-CSF. Week 10: * $p < 0.01$, SCF or SCF+G-CSF vs. saline or G-CSF; † $p < 0.05$, G-CSF vs. saline. **B:** Functional outcome examination by foot-fault test. Note that the foot-faults are found only after the induction of cortical brain ischemia but not before the induction of cortical brain ischemia, and SCF or SCF+G-CSF treatment significantly reduces the number of foot-faults in the affected forepaws. Week 1: * $p < 0.01$, SCF vs. all other 3 groups. Week 4: * $p < 0.01$, SCF+G-CSF vs. saline and G-CSF; † $p < 0.05$, SCF vs. saline; SCF+G-CSF vs. SCF. Week 7 and 10: * $p < 0.01$, SCF and SCF+G-CSF vs. saline and G-CSF. **C:** Infarction size determination. Note that infarction size is reduced by SCF and G-CSF alone or in combination. Reproduced from Zhao LR, Singhal S, Duan WM, Mehta J, Kessler JA. Brain repair by hematopoietic growth factors in a rat model of stroke. *Stroke*. 2007; 38:2584–2591, with permission of publisher.

Fig. 3.

SCF alone or in combination with G-CSF induces functional improvement when administered in chronic stroke. **A:** Schematic diagram of experimental design. **B:** Functional examination by limb placement test. **C:** Functional examination by foot-fault test. Note that only SCF+G-CSF induces stable and long-lasting functional improvement. SCF results in an unstable improvement, whereas G-CSF has no effect in functional recovery. * $p < 0.01$, # $p < 0.05$. Reproduced from Zhao LR, Berra HH, Duan WM, Singhal S, Mehta J, Apkarian AV, Kessler JA. Beneficial effects of hematopoietic growth factor therapy in chronic ischemic stroke in rats. *Stroke*. 2007; 38:2804–2811, with permission of publisher.

Fig. 4.

Neuronal activity in the somatosensory cortex of both hemispheres when stimulating the left affected forepaw. Note that only SCF+G-CSF treatment causes a significant increase in neuronal activity in the right hemisphere as compared to the left hemisphere. # $p < 0.05$. Reproduced from Zhao LR, Berra HH, Duan WM, Singhal S, Mehta J, Apkarian AV, Kessler JA. Beneficial effects of hematopoietic growth factor therapy in chronic ischemic stroke in rats. *Stroke*. 2007;38:2804–2811, with permission of publisher.

Fig. 5.

Angiogenesis and neurogenesis by SCF+G-CSF in chronic stroke. **A–C:** SCF+G-CSF augments bone marrow-derived endothelial cells in the brain. Green: bone marrow-derived cells (GFP⁺ cells). Red: endothelial cells (CD31⁺ cells). Blue: nuclear counterstaining (DAPI). **D–F:** SCF+G-CSF increases blood vessel density in the cortex of peri-infarct area. **G–I:** Bone marrow-derived neuronal like cells are increased by SCF+G-CSF. Arrowheads, bone marrow-derived neuronal like cells (yellow, NeuN⁺/GFP⁺ cells); arrows, bone marrow-derived cells (green). Blue: nuclear counterstaining (DAPI). * $p < 0.05$, ** $p < 0.01$. St, striatum; CC, corpus callosum; Inf, infarct cavity. Reproduced from Piao CS, Gonzalez-Toledo ME, Xue YQ, Duan WM, Terao S, Granger DN, Kelley RE, Zhao LR. The role of stem cell factor and granulocyte-colony stimulating factor in brain repair during chronic stroke. *J Cereb Blood Flow Metab.* 2009;29:759–770, with permission of publisher.

Fig. 6. SCF+G-CSF increases neuronal network formation. **A–C:** A 64% increase in mushroom-type dendritic spines is induced by SCF+G-CSF treatment in chronic stroke (Golgi staining and data analysis was provided by Neurostructural Research Labs, Inc). **A:** A pyramidal neuron in the cortex adjacent to infarct cavity by Golgi staining. **B:** Dendritic spines by Golgi staining. **D–F:** Dendritic branching in the peri-infarct cortex is increased by SCF+G-CSF treatment in chronic stroke. Red, MAP2 immunohistochemistry staining for dendrites. **G–I:** SCF+G-CSF increases neurite outgrowth and network formation in primary cortical neuron cultures (Piao *et al.*, unpublished observation). ** $p < 0.01$.

Table 1

Therapeutic Benefits of SCF and G-CSF on Brain Ischemia in Animal Models

Treatment	The Phase of Stroke	References
SCF	Acute phase	Zhao <i>et al.</i> , 2007 []
G-CSF	Acute phase	Schabitz <i>et al.</i> , 2003 []
G-CSF	Acute phase	Shyu <i>et al.</i> , 2004 []
G-CSF	Acute phase	Schneider <i>et al.</i> , 2004 []
G-CSF	Acute phase	Komine-Kobayashi <i>et al.</i> , 2004 []
G-CSF	Acute phase	Zhao <i>et al.</i> , 2007 []
SCF+G-CSF	Acute phase	Zhao <i>et al.</i> , 2007 []
SCF+G-CSF	Acute phase	Toth <i>et al.</i> , 2008 []
SCF+G-CSF	Subacute phase	Kawada <i>et al.</i> , 2006 []
SCF+G-CSF	Chronic phase	Zhao <i>et al.</i> , 2007 []
SCF	Chronic phase	Zhao <i>et al.</i> , 2007 []

Table 2

Patents for Use of SCF and/or G-CSF to Treat Stroke

Treatment	Title of patent	Inventors	Publication No.	References
G-CSF	G-CSF mimetics	Luengo, Juan I Duffy, Kevin J	US6346531	Luengo <i>et al.</i> , 2002 [53]
SCF or G-CSF	Therapeutic application of g-scf	Moukoko, Didier Pourquier, Didier	EP1465653A1	Moukoko <i>et al.</i> , 2004 [54]
SCF or G-CSF	Therapeutic application of SCF	Moukoko, Didier Pourquier, Didier	EP2277529A1	Moukoko <i>et al.</i> , 2011 [55]
SCF and G- CSF alone or in combination	Use of SCF and G-CSF in the treatment of cerebral ischemia and neurological disorders	Zhao, Li-Ru, Kessler, John, Singhal, Seema, Jayesh, Mehta	WO2006055260 Same as: US2005039792 US20100286039 EP1817047B1 EP1817047A2 AU2005306894 CA2586365	Zhao <i>et al.</i> , 2006 [56]
G-CSF	Use of g-csf for the treatment of stroke	Koch, Winfried, Laage, Rico, Schneider, Armin, Vogt, Gerhard	EP2036571A1 Same as: US20100284926 EP2195014A2 EP2412381A1 EP2195014B1	Koch <i>et al.</i> , 2009 [57]
G-CSF	Methods of treating neurological Conditions with hematopoietic growth factors	Schaebitz, Wolf-Ruediger; Schneider, Armin; Krueger, Carola; Sommer, Clemens; Schwab, Stefan; Kollmar, Rainer; Maurer, Martin; Weber, Daniela; Gassler, Nikolaus	US20090087481 Same as: US8071543B2	Schaebitz <i>et al.</i> , 2009 [58]
G-CSF	Use of G-CSF for treating ischemia	Franz, Wolfgang-Micheal Engelmann, Markus Georg Steinbeck, Gerhard	US20100209383	Franz <i>et al.</i> , 2010 [59]
G- CSF	Use of G-CSF for the extension of the therapeutic time-window of thrombolytic stroke therapy	Fisher, Marc, Schneider, Armin	US20120070403	Fisher <i>et al.</i> , 2012 [60]