Neuroprotective Effect of Granulocyte Colony–Stimulating Factor After Focal Cerebral Ischemia

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Background and Purpose—The potential neuroprotective effect of the granulocyte colony–stimulating factor (G-CSF) after glutamate-induced excitotoxicity in cell culture and after focal cerebral ischemia in rats was studied. We hypothesized the existence of the G-CSF receptor (G-CSFR) as a main G-CSF effector on neurons, and immunohistochemistry, immunoblotting, and polymerase chain reaction were performed. The G-CSFR–mediated action was studied by activation of signal transducer(s) and activator(s) of transcription-3 (STAT3) in the periphery of the infarction.

Methods—Neuroprotection of various G-CSF concentrations on glutamate-induced excitotoxicity was studied in cell culture. In vivo, ischemia was induced by use of a suture occlusion model of the middle cerebral artery (90-minute occlusion) in the rat. Thirty minutes after the induction of ischemia, the animals (n=12 per group) received G-CSF at 60 μg/kg body wt IV for 90 minutes or vehicle (saline). Infarct volume was calculated on the basis of 2,3,5-triphenyltetrazolium chloride staining 24 hours after ischemia. Expression of the G-CSFR was studied by immunohistochemistry and verified by reverse transcription–polymerase chain reaction and immunoblotting. Expression of STAT3 was determined by immunohistochemistry.

Results—In cell culture, G-CSF exhibited a significant neuroprotective effect after glutamate-induced excitotoxicity (P<0.05). A G-CSF concentration of 10 ng/mL was maximally effective, resulting in a nearly complete protection. In vivo, G-CSF reduced infarct volume to 47% (132.0±112.7 mm³ versus 278.9±91.6 mm³ [P<0.05] in the control group). Immunohistochemistry, Western blotting, and reverse transcription–polymerase chain reaction revealed the existence of G-CSFRs in neurons and glial cells. Animals treated with G-CSF significantly upregulated STAT3 in the periphery of the infarction compared with control animals (P<0.05).

Conclusions—G-CSF achieved a significant neuroprotective effect in cell culture and after intravenous administration after stroke. Increased STAT3 expression in the penumbra of G-CSF–treated rats suggests mediation by G-CSFR. (Stroke. 2003;34:745-751.)

Key Words: colony-stimulating factor, granulocyte excitotoxicity growth factors ischemia neuroprotection

Growth factors are polypeptides essentially involved in regulating survival, proliferation, maturation, and outgrowth of developing neuronal cells. Many factors display endogenous neuroprotective and neurotrophic effects.1–4 Such effects have also been reported with exogenous administration after brain trauma and stroke.4–6 After binding to high-affinity membrane receptors, the effects of growth factors are mediated by the activation of a series of kinases that translocate to the nucleus to phosphorylate transcription factors.7 Cells then can be induced to grow and differentiate or to obtain enough trophic support to survive.

Granulocyte colony–stimulating factor (G-CSF), a 20-kDa protein, is a member of the cytokine family of growth factors, along with tumor necrosis factor-α (TNF-α) and the interleukins. G-CSF stimulates the proliferation, survival, and maturation of cells committed to the neutrophilic granulocyte (NG) lineage through binding to the specific G-CSF receptor (G-CSFR).8 G-CSFR–mediated signaling activates the family of signal transducer(s) and activator(s) of transcription (STAT) proteins that translocate to the nucleus and regulate transcription.9 G-CSF is typically used for the treatment of different kinds of neutropenia in humans, but it may also have trophic effects on neuronal cells.10 However, nothing is known about the role of G-CSF during brain ischemia in vivo. This could be of interest, because G-CSF is one of the few growth factors approved for clinical use.

Therefore, we studied a potential neuroprotective effect of G-CSF after glutamate-induced excitotoxicity in cell culture as well as after focal cerebral ischemia in the rat. Furthermore, we hypothesized the existence of a neuronal G-CSFR.
as a main G-CSF effector, and immunohistochemistry, immunoblotting, and polymerase chain reaction (PCR) were performed. G-CSFR-mediated action was studied by activation of STAT3 in the periphery of the infarction (penumbra).

Materials and Methods

Cell Culture Experiments
Cerebellar granule cells were prepared from P7 mice. Freshly digested cerebella were disaggregated by trituration in the presence of 10 mg/mL trypsin and 5 mg/mL DNase. Cells were plated on poly-L-lysine–precoated 96-well plates (Sarsted). Cells were seeded at a density of 200,000 cells per well in basal modified Eagle medium (BME, GIBCO-BRL) supplemented with 10% fetal calf serum (PAA, Colbe), 20 mmol/L KCl, 50 IU/mL penicillin, and 50 μg/mL streptomycin. After 48 hours in culture, cytosine-d-arabinofuranoside (10 μmol/L) was added to arrest the growth of nonneuronal cells. On the day 7 in culture, cells were treated with glutamate stimulation. After another 4 hours at 37°C, cells were lysed by 1% SDS. The formazan product was quantified as the optical density of samples at 590 nm.

Focal Cerebral Ischemia
Experimental protocols were approved by the local ethics committee. Twenty-four male Wistar rats (Charles River, Sulzfeld, Germany) weighing 280 to 320 g were randomly assigned to the following groups: A (control group, n = 12), B (G-CSF group, n = 12). G-CSF [Neupogen, Amgen], dissolved in 2 mL of 0.9% saline for 90 minutes beginning 30 minutes after vessel occlusion. Rats had free access to food and water before the experiments.

Animals were anesthetized with an intraperitoneal injection of 100 mg/kg body weight ketamine hydrochloride (WDT). Anesthesia was maintained with 50 mg/kg body wt if necessary. A PE-50 polyethylene tube was inserted into the right femoral artery for continuous monitoring of mean arterial blood pressure, blood gases, hematocrit, leukocyte count, and blood glucose levels. The right femoral vein was cannulated by a PE-50 tube for treatment infusion. During the experiment, rectal temperature was monitored and maintained at 37°C by a thermostatically controlled heating pad (Führ Medical Instruments). Transient focal cerebral ischemia for 90 minutes was induced by using the suture occlusion model as recently published and described in detail by ZeaLonga et al. Sham-operated animals underwent the same experimental procedures, but the nylon filament was not advanced beyond the common carotid artery, so that no infarction occurred. After surgery, the catheters were removed, and the animals were allowed to recover from anesthesia and were given food and water ad libitum.

Measurement of Regional CBF
Laser-Doppler flowmetry (LDF, Periflux 4001 Master, Perimed AB) was used to monitor cerebral blood flow (CBF) before, during, and after occlusion of the middle cerebral artery (MCA). After the rats were placed in a stereotactic frame, their skulls were exposed, and a hole of 1.5-mm diameter was drilled under the microscope on the right side, 4 mm lateral and 2 mm caudal to the bregma. The dura was left intact, and the LDF probe (1.4 mm in diameter) was placed into the burr hole. The area selected for CBF monitoring corresponded to the territory of the occluded MCA.

Infarct Volume Calculation
Twenty-four hours after MCA occlusion, the rats were anesthetized with ketamine (150 mg/kg body wt) and decapitated. Their brains were dissected and cut into 5 coronal slices of 2-mm thickness, incubated in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C for 30 minutes, and immersion-fixed in a 10% buffered formalin solution. TTC-stained sections were photographed using a charge-coupled device camera (EDV-1000HR Computer Camera, Electrim Corp), and the investigator (R.K.) blinded to the study protocol measured the infarct sizes with a computerized image analyzer (Bio Scan Optimas). To compensate for the effect of brain edema, the corrected infarct volume was calculated as previously described in detail: corrected infarct area = left hemisphere area – right hemisphere area – infarct area. Infarct volumes were expressed as a percentage of the contralateral hemisphere.

Immunohistochemistry
A 2-mm-thick brain slice at the anterior commissure was immersion-fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer for 24 hours (n = 5 per group). After paraffin embedding, 1-μm-thick sections were cut and used for morphological analysis.

Immunohistochemical studies were performed with well-characterized antisera against myeloperoxidase (MPO, DAKO), glial fibrillary acidic protein (GFAP, DAKO), G-CSFR (Santa Cruz Biotechnology Inc), and STAT3 (Santa Cruz Biotechnology Inc). For antigen retrieval, sections provided for G-CSFR and STAT3 immunohistochemistry were heated for 20 minutes in a 10 mmol/L citrate buffer at 90°C. Sections were then incubated in normal swine serum (10% in PBS) for 30 minutes, followed by the primary antisera overnight at 4°C. Primary antibodies were diluted 1:150 (MPO), 1:400 (GFAP), 1:400 (G-CSFR), and 1:100 (STAT3), respectively. Immunoreactivity (IR) was visualized by the avidin biotin complex method (Vectastain, Vector Laboratories) as previously described. In a subset of control slides, preabsorption of the G-CSFR antiserum with the respective peptide did not produce immunostaining (Figure 3). When the primary antisera were omitted, no immunostaining was produced either (not shown).

Invasion of NGs was quantitatively measured by counting NGs per infarcted hemisphere. STAT3 protein expression was quantified in 2 overlapping fields rostrocaudal in healthy tissue adjacent to the infarction of the parietal cortex and the corresponding contralateral side (magnification × 400). To this end, neurons with nuclear translocation were counted, given as a percentage of STAT3-positive neurons from all neurons.

Western Blots
For immunoblotting, brain tissue (transient ischemia of 90 minutes) was lysed in 20 volumes (wt/vol) of homogenization buffer (320 mmol/L sucrose, 0.1 mmol/L phenylmethylsulfonyl fluoride, and 2 μg/mL pepstatin) at 4°C. Homogenates were centrifuged at 9200g for 15 minutes at 4°C. After the pellets were resuspended in 1/10 of the homogenization volume, aliquots for protein determination (Bio-Rad protein assay) were separated, and samples were rapidly frozen in liquid nitrogen and stored at −70°C. Each lane on an 8% SDS-polyacrylamide gel containing 4 mol/L urea was loaded with 15 μg protein and electrophoresed under standard conditions. Proteins were electrophoretically transferred to Immobilon-P membranes (Millipore Corp) by semidyblotting. After blocking in 3% nonfat dry milk in TBST (20 mmol/L Tris base, pH 7.6, 137 mmol/L NaCl, and 0.05% Tween 20) for 1 hour at room temperature, membranes were incubated with the primary G-CSFR antibody (1:500) overnight at 4°C. After washing in TBST the membranes were incubated for 1 hour at room temperature with 1:2000 dilutions of the appropriate horseradish peroxidase–conjugated secondary antibody. Immunoreactive bands were visualized in the linear range with enhanced chemiluminescence (Amersham Intl).

PCR for G-CSFR
Nonischemic mice were deeply anesthetized and perfused transcardially, and their brains were rapidly dissected. RNA was extracted from the brains by the RNA-clean kit (AGS), according to the manufacturer’s instructions. A total of 10 μg RNA was transcribed with MMLV reverse transcriptase and random hexamers. For PCR, the following primers from exons 5 and 7 of the murine G-CSFR were used: sense, 5'-CCC CTC AAA CCT ATG C-3';
Statistical Analysis
Values presented in the present study are mean±SD. After acquiring all data, the randomization code was broken. ANOVA and a subsequent post hoc Fisher protected least significant difference test or Bonferroni correction were used to determine the statistical significance of differences for in vitro data and physiological parameters. ANOVA and a subsequent post hoc t test were used for comparison of postmortem infarct volumes, MPO, and STAT3 immunohistochemistry. The χ² test was performed for mortality data. A value of *P*<0.05 was considered statistically significant.

**Results**

**Cell Culture Experiment**
Cell viability was measured by MTT metabolism, which relies on an intact mitochondrial function. Cerebellar granule cells that have been cultured for 7 days are known to die when they are exposed to glutamate. Pretreatment of cultures with G-CSF for 30 minutes reduced the excitotoxic effect of glutamate initially in a concentration-dependent manner. A G-CSF concentration of 10 ng/mL was maximally effective, resulting in a significant and nearly complete protection (Figure 1).

**Ischemia Experiment**
G-CSF achieved a potent neuroprotective effect after focal cerebral ischemia. Mean infarct volume in intravenous G-CSF–treated animals was 132.0±112.7 versus 278.9±91.6 mm³ in the control group or 9.96±8.3% (n=12) versus 22.7±6.7% of the total hemisphere (*P*<0.05, Figure 2).

G-CSF treatment significantly reduced mortality: 4 animals in the control group and 1 in the G-CSF–treated group died with sign of brain swelling and herniation within 24 hours of reperfusion (*P*<0.05). No statistical differences were observed between the control and G-CSF–treated groups for rectal temperature, pH, PCO₂, PO₂, hematocrit, blood glucose, heart rate, mean arterial pressure, and body weight for all animals (Table). Leukocyte count in the peripheral blood was significantly increased 24 hours after ischemia in G-CSF–treated compared with control animals (*P*<0.05, Table).

LDF monitoring revealed no statistical differences between the 2 treatment groups (data not shown).

**Immunohistochemistry**
MPO staining detected no NGs in the nonischemic hemispheres of both groups. MPO staining was not significantly different between G-CSF–treated animals and control animals on the basis of quantified MPO-positive cells in the ischemic hemisphere (14±17.6 versus 14.3±12.5, *P*=NS).

GFAP IR was present in scattered astrocytic processes throughout the cortex, striatum, and white matter of the noninfarcted hemisphere. No difference in the pattern and intensity of GFAP staining was detectable in the cortical peri-infarct zone in untreated or G-CSF–treated rats. In particular, GFAP IR was not increased in the cortical penumbra, either in the placebo group or in the G-CSF group (not shown). Within the infarct core, scattered GFAP immunoreactive astrocytes were detectable (not shown).

Immunohistochemically, staining for G-CSFR was detectable in scattered cortical neurons and neurites (Figure 3) in untreated and G-CSF–treated animals. Glial cells were also stained with the G-CSFR antibody (Figure 3). In the infarct core, no G-CSFR immunoreactive cells were seen. No difference in the pattern and intensity of G-CSFR IR was evident between the 2 experimental groups.

STAT3 IR was seen in scattered nuclei of neurons and glial cells within the uninfarcted hemisphere of placebo-treated and G-CSF–treated rats. Some cytoplasmic staining was also present in a few scattered neurons. STAT3 protein expression was significantly increased in the penumbra of the infarction in G-CSF–treated rats compared with untreated control rats (34.4±7.1% versus 13.7±4.4%, *P*<0.0003; Figures 4 and 5). No difference occurred on the contralateral side (16.2±6.9% versus 13.3±6.9%, *P*=NS).

**Wester Blot and PCR**
Using a reverse transcription (RT)-PCR specific for mouse G-CSFR, G-CSFR mRNA was detected in the brain tissue. The
Furthermore, STAT3 expression was significantly increased in neurons of the penumbra in G-CSF–treated animals and control animals. Neutrophils exhibited specific binding of G-CSFR antiserum, indicative of a G-CSFR. Interaction with this receptor activates the Janus family kinases (JAKs) and STATs. JAKs are nonreceptor–mediated after binding to the high-affinity neurotrophin receptor G-CSFR. Because the presence of G-CSFR on neurons is novel, we verified this finding by Western blot and RT-PCR. The results of the present study demonstrate that G-CSF is neuroprotective in vitro (nearly complete protection) and neuroprotective in vivo (47% infarct-reducing effect after systemic application). What could be the mechanism of G-CSF–mediated neuroprotection after stroke? Glutamate-induced neurotoxicity and may therefore have anti-excitotoxic effects after ischemia. The main mechanism is thought to be mediated after binding to the high-affinity neurotrophin receptor G-CSFR. Interaction with this receptor activates the Janus family kinases (JAKs) and STATs. JAKs are nonreceptor–type tyrosine protein kinases that become activated on ligand-induced receptor dimerization. G-CSF–induced activation of JAK phosphorylates STAT on a conserved tyrosine residue, which induces STAT dimerization. Furthermore, STAT translocates to the nucleus and subsequently regulates gene expression. STAT3 is the principal STAT protein acti-

### Physiological Parameter of G-CSF Treated Animals and Controls

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Rectal Temperature</th>
<th>pH</th>
<th>Pco2, mm Hg</th>
<th>P02, mm Hg</th>
<th>Hct, %</th>
<th>Glucose, mg/dL</th>
<th>MABP, mm Hg</th>
<th>HR, beats/min</th>
<th>Leukocytes, ×10^9/L</th>
<th>Body Weight, g</th>
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<td>Pre-ischemia</td>
<td>Control</td>
<td>37 ± 0.2</td>
<td>7.38 ± 0.03</td>
<td>39 ± 7</td>
<td>89 ± 7</td>
<td>47.4 ± 3.6</td>
<td>263 ± 25</td>
<td>98 ± 12</td>
<td>358 ± 13</td>
<td>1.9 ± 0.3</td>
<td>314 ± 25</td>
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<td>rG-CSF</td>
<td>37 ± 0.3</td>
<td>7.35 ± 0.02</td>
<td>38 ± 5</td>
<td>91 ± 7</td>
<td>46.0 ± 0.9</td>
<td>251 ± 31</td>
<td>102 ± 15</td>
<td>350 ± 24</td>
<td>1.8 ± 0.4</td>
<td>318 ± 29</td>
</tr>
<tr>
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<td>Control</td>
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<td>7.38 ± 0.02</td>
<td>41 ± 6</td>
<td>88 ± 5</td>
<td>45.3 ± 0.8</td>
<td>160 ± 13</td>
<td>112 ± 21</td>
<td>384 ± 16</td>
<td>6.5 ± 0.6</td>
<td>320 ± 30</td>
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<tr>
<td></td>
<td>rG-CSF</td>
<td>37 ± 0.2</td>
<td>7.37 ± 0.03</td>
<td>39 ± 4</td>
<td>89 ± 8</td>
<td>44.3 ± 0.7</td>
<td>172 ± 17</td>
<td>109 ± 19</td>
<td>371 ± 27</td>
<td>6.8 ± 0.3</td>
<td>324 ± 32</td>
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<td>7.39 ± 0.03</td>
<td>37 ± 3</td>
<td>87 ± 8</td>
<td>44.6 ± 0.8</td>
<td>149 ± 12</td>
<td>101 ± 14</td>
<td>368 ± 13</td>
<td>8.2 ± 0.4</td>
<td>320 ± 30</td>
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<td>39 ± 6</td>
<td>89 ± 5</td>
<td>44.2 ± 0.4</td>
<td>152 ± 14</td>
<td>99 ± 8</td>
<td>372 ± 9</td>
<td>8.5 ± 0.3</td>
<td>324 ± 32</td>
</tr>
<tr>
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<td>7.38 ± 0.02</td>
<td>38 ± 5</td>
<td>91 ± 5</td>
<td>43.3 ± 0.9</td>
<td>133 ± 7</td>
<td>102 ± 16</td>
<td>366 ± 17</td>
<td>9.7 ± 0.8</td>
<td>320 ± 30</td>
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<tr>
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<td>39 ± 7</td>
<td>89 ± 9</td>
<td>42.8 ± 1.1</td>
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<td>120 ± 17</td>
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<td>86 ± 6</td>
<td>46.7 ± 1.5</td>
<td>198 ± 13</td>
<td>115 ± 17</td>
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<td>*3.8 ± 0.8</td>
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<td>204 ± 16</td>
<td>117 ± 21</td>
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<td>293 ± 16</td>
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</tbody>
</table>

Values are given as mean ± SD (P<0.05; ANOVA; F test).
Hct indicates hematocrit; MABP, mean arterial blood pressure; HR, heart rate.

PCR product had the expected size of 567 bp. The identity was verified by sequencing the PCR product (Figure 6).

In immunoblotting experiments with cortical extracts (Figure 7), the G-CSFR antiserum detected a protein band of ~130 kDa, consistent with the deduced molecular weight. In addition, a few bands of lower molecular weight were seen, probably reflecting breakdown products. After preabsorption of the G-CSFR antiserum with the respective peptide, the bands disappeared (Figure 7).

### Discussion

The results of the present study demonstrate that G-CSF is neuroprotective in vitro (nearly complete protection) and displays a significant (47%) infarct-reducing effect after stroke and after intravenous administration. Neurons in the periphery of the infarction but also on the contralateral side exhibited specific binding of G-CSFR antiserum, indicative of a G-CSFR. Because the presence of G-CSFR on neurons is novel, we verified this finding by Western blot and RT-PCR. Furthermore, STAT3 expression was significantly increased in neurons of the penumbra in G-CSF–treated animals compared with control animals, suggesting an increased sensitivity to G-CSF. There was no effect on CBF as measured by LDF when both groups were compared. There were no significant differences in physiological parameters or in weight decline between both groups during the experiment. Mortality rate was significantly improved in animals treated with G-CSF compared with control animals. Neutrophilic blood count was significantly increased after 24 hours in G-CSF–treated animals compared with control animals. MPO staining as a measure of invading NGs into the ischemic hemisphere was not significantly different between G-CSF–treated animals and control animals.

The dose of the intravenously delivered G-CSF (60 μg/kg body wt) used in the present study was adjusted to the results of the in vitro experiment and was comparable to the doses used for other experimental conditions. It had been tested for safety in a pilot project, and no significant side effects were observed. However, this dose (60 μg/kg body wt) is 6 times higher than the approved dose for treatment of human myelodysplastic syndrome and other diseases.

An infarct reducing effect of 50% achieved with G-CSF is comparable to that of other growth factors, such as basic fibroblast growth factor (bFGF) and insulin-like growth factor, after systemic application. What could be the mechanism of G-CSF–mediated neuroprotection after stroke? Glucose deprivation and excitotoxicity with subsequent Ca2+ overload of cells as well as apoptosis and decreased energy reserve in the face of increased requirements (eg, from spreading depression) are the main causes of neuronal cell death after ischemia.

As shown in the present study, G-CSF protects in vitro neuronal cells against glutamate-induced neurotoxicity and may therefore have anti-excitotoxic effects after ischemia. The main mechanism is thought to be mediated after binding to the high-affinity neurotrophin receptor G-CSFR. Interaction with this receptor activates the Janus family kinases (JAKs) and STATs. JAKs are nonreceptor–type tyrosine protein kinases that become activated on ligand-induced receptor dimerization. G-CSF–induced activation of JAK phosphorylates STAT on a conserved tyrosine residue, which induces STAT dimerization. Furthermore, STAT translocates to the nucleus and subsequently regulates gene expression. STAT3 is the principal STAT protein acti-
vated by G-CSFR. STAT3 mediates antiapoptotic function by activating bcl-2, and it induces proliferation and differentiation of granulocytes by upregulating the c-myc gene. As shown in the present study by use of immunohistochemistry, RT-PCR, and Western blot, G-CSFR exists not only on hematopoietic cells but also on neurons and glial cells. Furthermore, in G-CSF–treated animals, STAT3 is upregulated in neurons of the penumbra, which in turn may mediate antiapoptotic effects, such as bcl-2 upregulation, as shown for brain-derived neurotrophic factor or bFGF, and provides

![Figure 3](image3.png)

Figure 3. Within the nonischemic cortex, numerous scattered neurons (arrow) and their neurites (arrowhead) exhibit G-CSFR IR (a, original magnification ×200). Preabsorption of the antiserum with the respective peptide results in no immunostaining in the corresponding area (b). G-CSFR immunostained glial cells in the corpus callosum are shown (c, original magnification ×400).

![Figure 4](image4.png)

Figure 4. Immunohistochemistry with an antibody against STAT3: Quantified were cortical neurons in the unaffected contralateral side (CL) and ipsilateral in the vicinity of the infarction (IL) in G-CSF–treated and control animals. Note the significant upregulation of STAT3-positive neurons adjacent to the infarction in the G-CSF–treated group. *P<0.05 by t test.

![Figure 5](image5.png)

Figure 5. STAT3 immunohistochemistry. Note that numerous neuronal nuclei are positively stained in the cortical penumbra of G-CSF–treated rats (b, arrows) compared with the cortical peri-infarct area of a placebo-treated animal (a, arrows; left, infarct; right, penumbra; original magnification ×200).
trophic support of neurons to survive. Dense nuclear labeling of STAT3 in the penumbra could reflect membrane receptor-mediated translocation of STAT3 from the cytoplasm to the nucleus, which has already been shown for activated microglia after cerebral ischemia.20

G-CSF is known to stimulate release, enhancement of effector function, and extension of the life span by delaying apoptotic cell death of NGs, the body’s first line of defense against all kinds of infections.9 Neutrophils could occlude microvessels, and subsequent invasion of leukocytes triggers the release of proteolytic enzymes, oxygen-derived free radicals, interleukins, and TNF-α, effects that are known to deteriorate infarct size and outcome after cerebral ischemia.21–23 In contrast, G-CSF has significant anti-inflammatory effects: in models of peripheral infections, G-CSF-induced JAK-STAT signaling reduces TNF-α, interleukin (IL)-1β, IL-2, IL-6, and IL-8 and elevates IL-1β receptor antagonists.24–29 G-CSF decreases TNF-α release in vitro and in vivo in healthy volunteers and elevates levels of antagonists for TNF, IL-6, IL-8, and IL-1β.9,24,25 This could reduce cytokine toxicity, neutrophil activation, and infiltration. Indeed, reduced neutrophil infiltration in lung and ileum has been observed in a model of splanchic ischemia and reperfusion 15 minutes after the administration of G-CSF and reperfusion of the small bowel.29 In accordance with these findings, we did not observe in the present study an increase of neutrophil infiltration into the ischemic hemisphere, although total neutrophilic blood count increased after G-CSF treatment. Reduced cytokine toxicity may also limit interactions between neutrophils and the endothelium in microvessels and improve microvascular flow in the penumbra.22

G-CSF treatment in the present study did not affect CBF as measured by LDF. However, LDF measures blood flow in a single location, and it cannot be discerned whether microvascular flow in the penumbra of the infarction may have improved with G-CSF treatment. This may require further investigation because another growth factor, bFGF, has been shown to dilate collaterals in the peri-ischemic zone, even at doses not promoting systemic hypotension, thus increasing blood flow to the penumbral regions.20

In conclusion, intravenous administration of G-CSF achieved a significant neuroprotective effect in cell culture and after focal cerebral ischemia and was associated with increased STAT3 expression. These effects were probably mediated by interaction with G-CSFR. Clearly, further studies are necessary to clarify the phenomenon and mechanisms of neuroprotection by G-CSF after focal cerebral ischemia.

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References


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