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Granulocyte-colony stimulating factor for acute ischemic stroke: a randomized controlled trial of safety and efficacy (STEMTHER)

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Abstract

Background and Purpose. Granulocyte-colony stimulating factor (G-CSF) is a neuroprotector and a stimulator of autologous bone marrow stem cell release can, in theory, improve the outcome of acute ischemic stroke. We sought to examine the safety and efficacy of using G-CSF (Leukostim®) to treat acute ischemic stroke.

Methods. We conducted a single-centre, unmasked, randomized controlled trial STEMTHER (NCT00901381) involving twenty adult patients with unilateral ischemic stroke in the carotid region presenting within forty-eight hours of onset. The experimental group (n=10) received subcutaneous G-CSF injections (10 mkg/kg per day) combined with conventional therapy for five days. The primary outcome was the degree of dependence in daily activities measured by the modified Rankin Scale obtained at 180 days. Safety was evaluated by frequency of hemorrhagic transformation of infarction and serious adverse events.

Results. All of the patients in the experimental group completed the five-day course of treatment. Four of them (three in the control and one in the experimental group) were lost to follow-up. Analysis of efficacy included data on patients who had completed follow-up. Analysis of safety included data on all patients. The experimental and control groups showed no statistical difference in neurological impairment or degree of disability and dependence at 180 days.

Conclusions. Acute ischemic stroke therapy with G-CSF (10 mkg/kg per day) for five days is safe, but does not improve the outcome at 180 days after stroke onset. Further investigation of high-dose G-CSF efficacy is needed.

Stroke is the second leading cause of death and disability worldwide [1]. Therefore, finding effective methods to protect and recover damaged brain cells has been challenging for different research groups. The use of granulocyte-colony stimulating factor (G-CSF) is potentially important in the treatment of stroke [2]. G-CSF has a number of positive effects [3], such as antiapoptotic, anti-inflammatory, excitoprotective and neurotrophic; it also drives angio- and neurogenesis. The administration of G-CSF mobilizes CD34-positive cells (CD34+) hematopoietic stem cells from the bone marrow into peripheral blood [4], promoting neuronal repair and recovery of brain function [5]. Meta-analysis of the efficacy of G-CSF in animal models of focal cerebral ischemia showed that G-CSF reduces infarct volume and enhances functional recovery [6].

Ischemic stroke or acute myocardial infarction triggers the mobilization of hematopoietic stem/progenitor CD34+ cells from the bone marrow into peripheral blood [7]. Therefore, the administration of exogenic G-CSF to enhance natural mechanisms of protection and repair seems reasonable. However, we should bear in mind possible complications of the therapy [8]. According to data from a meta-analysis [9] the use of G-CSF for the treatment of acute myocardial infarction was found to be safe but ineffective. Recent studies (unknown to our research group at the beginning of the study) including randomized clinical trials of the efficacy and safety of G-CSF for acute ischemic stroke [10–12] have shown that G-CSF could be safe; however, the data on efficacy is conflicting.

In this study, we sought to determine the safety and efficacy of G-CSF therapy for acute stroke.

Materials and Methods

We conducted a prospective, single-center, unmasked, randomized controlled trial of the safety and efficacy of G-CSF therapy in patients with acute ischemic stroke. The protocol was

approved by the Ethics Committee of Federal Drug Quality Control Commission (Protocol № 85 2007/02/27), the Ministry of Health and Social Development of the Russian Federation (Permission № 209 2007/05/21) and meets the Declaration of Helsinki and the National Standard of the Russian Federation "Good clinical practice" (GOST R 52379-2005). This study is registered in the clinical trials database www.clinicaltrials.gov, the registration number is NCT00901381.

Subjects

We recruited adult patients 40 – 70 years of age with unilateral ischemic stroke in the carotid region, impaired consciousness (the Glasgow coma scale [GCS]: scores range from 8 to 15) and hemiparesis (Medical Research Council scale [MRC] <5) who presented within 48 hours of onset to the City Clinical Hospital №40. The principal exclusion criteria included premorbid dependency (modified Rankin Scale [mRS] >0), hemorrhagic stroke, coagulopathy, malignancy and pregnancy. Full written informed consent was obtained from patients before randomization, or assent was received from a relative/caregiver.

Randomization

Randomization was carried out in the following manner: we prepared 20 envelopes containing cards with "experimental group" or "control group" inscribed. After obtaining a patient's informed consent, the physician responsible for the patient selected an opaque envelope that contained the group assignment.

Blinding

Randomization results were known to the examining physician evaluating neurological state and functional outcome and to the patient. However, a radiologist using magnetic resonance imaging (MRI) to evaluate patients' scans was blinded to randomization results.

Therapy

We used genetically engineered recombinant human G-CSF (Leukostim®, Biocad, Russia), which has physicochemical characteristics and specific biological activity identical to the widely used drug Neupogen® (F. Hoffman-La Roche Ltd., Swizerland; international nonproprietary name: filgrastim).

According to randomization, the G-CSF group received subcutaneous recombinant human G-CSF injections (10 mkg/kg per day in the morning) combined with conventional therapy for stroke for five days. The course of injections was abbreviated in case of: (1) leukocytosis (scores > 50×10^9 cells/L); (2) hemorrhagic transformation of infarction; (3) serious adverse events. The other group received conventional therapy for stroke approved by the Ministry of Health and Social Development of the Russian Federation (Order № 513 2007/08/01).

Objective

We compared the safety and efficacy of G-CSF (Leukostim®) combined with conventional medical therapy with the safety and efficacy of conventional medical therapy for acute ischemic stroke.

We hypothesized that the use of G-CSF for acute ischemic stroke would (1) reduce the degree of dependence in the daily activities, (2) decrease volume of cerebral infarction, (3) be safe.

Clinical evaluation

Therapy efficacy was evaluated according to the degree of dependence in the daily activities as measured by the modified Rankin Scale obtained at 0, 1, 3, 5, 7, 14, 30, 90, and 180 days. We performed neurological examination at 0, 1, 3, 5, 7, 14, 30, 90, and 180 days after patient enrollment to assess scores from the MRC scale for limbs of patients affected by hemiparesis, National Institutes of Health Stroke Scale (NIHSS), Barthel Index (BI) and Glasgow Outcome Scale (GOS). We evaluate consciousness at 0, 1, 3, 5, 7, and 14 days using GCS.

Safety was evaluated by mortality and frequency of hemorrhagic transformations and serious adverse events. We recorded clinical information on musculoskeletal pains, body temperature increase, injection site tenderness, thrombocytopenia, spleen enlargement, supraventricular tachyarrhythmia, vasculitis, diffuse edema, shortness of breath, pericardial effusion, nausea and vomiting.

Cerebral infarct volume

MRI was performed at 0, 1, 3, 14, 90, and 180 days using Magnetom Symphony 1.5 T (Siemens, Germany); this scan included sagittal T1-weighted images and axial PD-, T2-weighted, FLAIR images. To evaluate volume of brain infarction we used axial FLAIR sequence (TR 9000, TE 115, FOV 230, matrix 224×256, slices were 6 mm thick) with the subsequent mathematical data analysis. We used DWI images and diffusion maps to determine precisely the acute stage of stroke at multi-focal encephalopathy. Wallerian degeneration (WD) was evaluated by using the following scores: 0 – no WD; 1 – WD in the part of brain stem at the site of primary lesion; 2 – WD of average extent; 3 – WD of entire brain stem.

Laboratory measures

Full blood counts were analyzed on a standard hematology analyzer at 0, 1, 3, 5, 7, 14, 30, 90 and 180 days.

The percentage of CD34+ in peripheral blood stem cell harvests was determined using two-color flow cytometry (FACS Canto, FACS Canto II; Becton & Dickinson, USA [BD]) at 0, 1, 3, 5 and 7 days. We used monoclonal antibodies (MoAb) against CD45 (2D1, BD) and CD34 antigens (8G12, BD) stained with fluorescein isothiocyanate (FITC) and R-phycoerythrin (PE), respectively. MoAb staining was done according to manufacturer's instructions. Whole blood was incubated with the cocktail of MoAbs. Samples were lysed using solution ("FACS Lysing solution", BD) then washed with phosphate-buffer saline ("Cell wash", BD), resuspended in 1 ml of PBS and analyzed by flow cytometry within two hours after staining. FACS machine settings and instrument stability monitoring were optimized by calibration system "7-color setup beads" (BD). At least 150000 CD45-positive events were acquired per tube. Analysis of immunophenotyping results was performed in FACS Diva 4.0-6.1 software (BD) according to international guidelines [13]. The result was calculated as percentage of CD34+ among all leukocytes (CD45-positive cells).

Biochemical measurements of bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), creatinine, urea, uric acid, crude protein, albumin, glucose, cholesterol, triglyceride, calcium, potassium, sodium, and chlorine were performed at 0, 3, 5, 7, 14 and 180 days using a standard biochemical analyzer.

Blood coagulation (at 0, 1, 3, 5, 7, 14 and 180 days) was measured using ROTEM® thromboelastometry (Pentapharm, Germany).

Statistical methods

Statistical data analysis was performed in SPSS software (for Windows®, version 16; SPSS Inc.). Parameters in our study did not demonstrate a Gaussian distribution. Moreover, since

the groups of patients were small, we applied exact non-parametric tests. We expressed quantitative variables as medians (interquartile range [IQR]) and qualitative variables as counts (percentage). Differences in the study population were analyzed using the Mann-Whitney U test or the Fisher test as appropriate. All tests were two-tailed, and a p value of less than 0.05 was used to define a statistically significant difference.

Results

Twenty patients were enrolled between June, 2007 and August, 2008 (Figure). Six patients completed the full course of G-CSF treatment. Four patients received an incomplete course of treatment (three of these patients developed hyperleukocytosis; and one developed hemorrhagic transformation of infarction). Four patients (three in the control group and one in the experimental group) were lost to follow-up not earlier than fourteen days post stroke onset. Analysis safety included data on all patients. However, analysis of efficacy included data on patients who completed the 180-day follow-up.

Clinical data

The baseline characteristics are presented in Table 1. Impairment (GCS, MRC, NIHSS), disability (BI, GOS), and dependence in daily activities (mRS) had no difference between the groups (Table 2).

Laboratory data

G-CSF increased the number of CD34+ in peripheral blood with the peak level occurring at day 5 (Table 3). The total leukocyte count increased in patients from the experimental group; most of this response was driven by increases in neutrophil count (data not shown). Erythrocyte counts did not change with G-CSF therapy. We recorded platelet count decrease

in patients from the experimental group at day 7; however, this response did not cause coagulation failure as both clinical and rheological data showed.

Bilirubin level at 3, 5, and 7 days and GGT level at day 3 was lower in the G-CSF group than in the control group (Table 4). There was no difference in other blood biochemistry parameters between the groups.

Cerebral infarct volume

No significant difference in cerebral infarct volume was observed between the groups (Table 5). Starting from the third day after stroke onset the G-CSF group showed paradoxical, but statistically insignificant increase of the relative size of the infarction zone (in percentage from the baseline characteristics). The evidence of WD on MRI scans in the G-CSF group (0 IQR [0 – 3]) was approximately equal to the evidence in the control group (0 IQR [0 – 0.5]) that was statistically insignificant (p=0.574).

Adverse events and complications

Mortality, frequency of hemorrhagic transformations and adverse events were comparable between the groups (Table 2). There was only one fatal outcome in the control group at day 8 after randomization caused by progressive ischemia. The only fatal outcome in the experimental group was registered after discharge from the hospital and was not connected with the received therapy (the cause of death was acute heart failure at day 28).

Clinical data on musculoskeletal pains, body temperature increase, injection site tenderness, spleen enlargement, supraventricular tachyarrhythmia, vasculitis, diffuse edema, shortness of breath, pericardial effusion, nausea, and vomiting connected with G-CSF therapy were not registered.

Discussion

We found no differences in functional outcome and infarct volume between the groups though this study was not large enough to show these differences. However, we found that G-CSF was safe in the studied dose and effective in mobilizing CD34+ stem cells from bone marrow into peripheral bloodstream in acute ischemic stroke. These results conform to the results from other studies [10–12], demonstrating that G-CSF is safe. However, drug efficacy was not demonstrated in two of the three trials.

How can we explain the absence of G-CSF efficacy in acute ischemic stroke? Firstly, it is possible that higher doses of the drug are required. In the study by Schäbitz et al. [10] one of the subgroups received the total dose of 180 mkg/kg during three days; nonetheless, G-CSF failed to show any difference from placebo (unpublished data, 2007). In their new trial, AXIS-2 (NCT00927836), the efficacy of a total dose of 135 mkg/kg injected over three days will be evaluated. Therefore, it is too early to make conclusions about G-CSF inefficiency in higher doses. Secondly, G-CSF inefficiency in our study can be explained by a single daily injection of the drug. It is known that twice daily administration is more preferable than once daily administration of the drug in similar total doses [14]. Thirdly, it can be caused by relatively late G-CSF treatment after stroke onset. Fourthly, it is probably necessary to enroll patients who benefit from this therapy. Thus, the study AXIS-2 will include patients only with infarct volume more than 15 cm^3.

An unexpected result in our study was that bilirubin and GGT levels were lower in the G-CSF group than in the control group. Usually, GGT level increases in ischemic stroke [15], and bilirubin level decreases only at ten days after onset [16]. It is also known that G-CSF can increase GGT but not bilirubin level in patients with chronic heart failure [17].

How can we explain our results? Firstly, the cause can be an artifact. Concerning GGT, we can assume the accidental character of the received data, as the difference between the groups was recorded only once. However, the changes in bilirubin levels were consistent as they were recorded repeatedly. Secondly, it is possible that G-CSF decreases toxic effects of other drugs on the liver, but in this case we would expect increases in ALT and AST levels. Thirdly, it is possible that we have detected a biochemical marker of G-CSF protection. One recent study showed an association between bilirubin and oxidative stress [18]. Referring to it we could assume that there is an association between G-CSF protection and bilirubin levels as a component of cerebral protection. GGT, another non-specific marker of hepatic dysfunction, could also be a marker of cerebral insults [15]. It is possible that G-CSF decreases the level of cerebral damage indicated by a reduction in markers such as bilirubin and GGT.

Currently we cannot explain the tendency to the increase of the relative size of infarction zone in the G-CSF group starting from the third day after the stroke onset. These data were statistically insignificant. However, we will investigate this tendency in further trials.

Summary

The results of our study confirm that the use of G-CSF in the standard therapeutic dose mobilizes stem cells from bone marrow into peripheral blood in patients with acute ischemic stroke. G-CSF appears safe but its efficacy remains unproved. Further investigation of the therapy with higher G-CSF doses is needed.

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Table 1. Baseline characteristics of patients by study group.

Factor	G-CSF n=10	Control n=10	p
Male	8 (80%)	7 (70%)	1.000
Age, yr	50 (46–57)	54 (45–57)	0.643
Left-hemisphere stroke	7 (70%)	7 (70%)	1.000
Glasgow Coma Scale	14 (13–14)	14 (13–14)	0.759
NIHSS	14 (12–19)	13 (8–15)	0.223
MRC for arm function	0 (0–0)	1 (0–2)	0.034
MRC for leg function	1 (0–3)	3 (1–3)	0.193
Barthel Index	5 (0–14)	13 (0–29)	0.272
mRS	5 (4–5)	4 (4–5)	0.465
Glasgow Outcome Scale	5 (5–5)	5 (5–6)	0.458

NIHSS – the National Institutes of Health Stroke Scale; MRC – the Medical Research Council scale; mRS – modified Rankin Scale.

Table 2. Data on drug efficacy and safety at 180 days.

Factor	G-CSF	Control	p
Efficacy			
mRS	2 (1–3)	2 (2–3)	0.889
mRS ≥2	5 (50%)	5 (50%)	1.000
Glasgow Coma Scale (at 14 days)	15 (14–15)	15 (15–15)	0.642
NIHSS	4 (1–7)	2 (2–5)	0.425
MRC for arm function	3 (3–5)	4 (3–4)	0.457
MRC for leg function	4 (4–5)	4 (4–5)	1.000
Barthel Index	90 (85–98)	100 (85–100)	0.233
Glasgow Outcome Scale	7 (7–8)	7 (7–7)	0.890
Safety			
Mortality	1 (10%)	1 (10%)	1.000
Hemorrhagic transformation	1 (10%)	1 (10%)	1.000
Leukocytosis > 50×10^9 cells/L	3 (30%)	0 (0%)	0.105
Other SAE	0 (0%)	0 (0%)	1.000

mRS – modified Rankin Scale; NIHSS – the National Institutes of Health Stroke Scale; MRC – the Medical Research Council scale; SAE – serious adverse events.

Table 3. Peripheral blood picture.

Parameter	Group	Day 0	Day 1	Day 3	Day 5	Day 7	Day 14
CD34+	G-CSF	0.02 (0.01–0.03)	0.01 (0.00–0.02)	0.05 (0.02–0.08)	0.09 (0.04–0.15)*	0.04 (0.01–0.14)	N/A
	Control	0.02 (0.01–0.02)	0.01 (0.01–0.02)	0.02 (0.01–0.04)	0.02 (0.01–0.04)	0.03 (0.01–0.03)	N/A
WBC	G-CSF	8.6 (6.8–10.3)	25.2 (16.8–38.8)*	39.4 (35.8–42.3)*	31.7 (18.4–36.6)*	15.9 (14.1–24.9)*	6.5 (5.5–7.2)
	Control	8.0 (6.8–10.6)	7.9 (7.3–9.2)	8.2 (5.7–9.5)	6.7 (5.8–9.1)	6.9 (5.7–8.2)	7.2 (6.2–8.0)
RBC	G-CSF	4.46 (4.06–5.15)	4.30 (4.04–4.97)	4.62 (3.78–5.14)	4.67 (4.24–5.30)	4.63 (4.24–4.98)	4.28 (3.88–4.70)
	Control	4.51 (4.09–4.96)	4.48 (4.29–4.88)	4.42 (4.01–4.88)	4.14 (4.00–4.59)	4.45 (4.25–4.63)	4.29 (4.11–4.96)
PLT	G-CSF	288 (106–541)	241 (87–381)	220 (165–429)	163 (134–357)	173 (118–262)*	345 (155–736)
	Control	316 (170–483)	307 (136–387)	287 (139–358)	300 (102–417)	314 (111–443)	331 (199–500)

* statistically significant difference between groups ($p<0.05$)

CD34+ – CD34-positive cells (percentage of among all leukocytes); WBC – white blood cell count (10^9 cells/L); RBC – red blood cell count (10^{12} cells/L); PLT – platelet count (10^9 cells/L); N/A – not analyzed.

Table 4. Blood biochemistry (significant parameters).

Parameter	Group	Day 0	Day 3	Day 5	Day 7	Day 14	Day 180
TB, mol/L	G-CSF	10.8 (9.1–11.6)	6.9 (6.3–10.6)*	7.7 (6.4–9.5)*	6.9 (5.5–9.7)*	8.8 (7.2–14.6)	10.5 (7.5–15.6)
	Control	11.2 (9.7–27.8)	13.5 (11.6–32.5)	11.6 (9.0–17.2)	11.1 (9.3–14.1)	9.4 (8.8–11.6)	10.1 (7.5–12.0)
GGT, U/L	G-CSF	43.2 (28.9–71.2)	42.5 (26.6–53.0)*	64.0 (46.1–115.3)	54.8 (48.7–119.5)	38.1 (31.3–81.0)	19.1 (15.0–70.6)
	Control	77.2 (34.4–127.5)	72.0 (52.7–90.0)	66.8 (54.3–90.6)	63.6 (33.8–106.4)	55.0 (36.5–93.2)	17.5 (11.6–38.0)
ALT, U/L	G-CSF	31.4 (13.5–65.8)	26.5 (17.0–51.0)	55.8 (26.9–73.5)	63.8 (35.8–79.1)	36.6 (24.7–59.4)	26.4 (14.3–51.9)
	Control	25.6 (20.7–30.0)	36.6 (23.0–77.0)	41.0 (32.6–103.0)	59.0 (30.5–90.0)	46.4 (26.0–60.1)	19.2 (11.7–30.0)
AST, U/L	G-CSF	31.0 (18.0–66.6)	28.3 (25.7–49.7)	52.0 (36.0–59.0)	50.6 (39.2–59.5)	37.3 (21.6–39.9)	25.8 (20.2–42.8)
	Control	29.9 (26.8–47.2)	43.0 (32.2–58.0)	51.8 (33.9–69.1)	40.1 (39.0–50.3)	34.0 (30.1–44.6)	25.8 (20.4–34.4)

* statistically significant difference between groups ($p<0.05$)

TB – total bilirubin; GGT - gamma-glutamyltransferase; ALT - alanine aminotransferase; AST - aspartate aminotransferase.

Table 5. Cerebral infarct volume.

	Absolute values, mm^3			Relative values, %		
Day	G-CSF	Control	p	G-CSF	Control	p
0	69.5 (13.0–138.8)	51.4 (14.5–109.2)	0.780	100	100	N/A
1	115.6 (66.1–165.8)	49.4 (33.8–157.0)	0.573	133.0 (110.8–315.0)	134.5 (116.0–150.3)	0.915
3	97.0 (58.7–246.1)	56.2 (35.5–133.9)	0.278	181.5 (95.8–392.3)	120.0 (101.0–156.0)	0.530
14	85.5 (44.4–219.2)	35.4 (24.3–101.5)	0.211	184.0 (81.3–323.0)	86.0 (79.8–118.0)	0.315
90	100.7 (30.9–183.4)	60.9 (20.8–136.6)	0.408	198.0 (75.0–416.5)	88.0 (76.0–104.0)	0.392
180	86.4 (31.6–145.8)	72.2 (14.4–142.5)	0.694	180.5 (80.0–510.0)	66.0 (66.0–109.0)	0.279

N/A – not analyzed.

Figure. Study design.