Phase I Study of Intravenous Low-dose Granulocyte Colony-stimulating Factor in Acute and Subacute Ischemic Stroke

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Background: Granulocyte colony-stimulating factor (G-CSF; filgrastim) may be useful for the treatment of acute ischemic stroke because of its neuroprotective and neurogenesis-promoting properties, but an excessive increase of neutrophils may lead to brain injury. We examined the safety and tolerability of low-dose G-CSF and investigated the effectiveness of G-CSF given intravenously in the acute phase (at 24 hours) or subacute phase (at 7 days) of ischemic stroke. *Methods:* Three intravenous dose regimens (150, 300, or 450 μg/body/day, divided into 2 doses for 5 days) of G-CSF were examined in 18 patients with magnetic resonance imaging (MRI)–confirmed infarct in the territory of the middle cerebral artery. Nine patients received the first dose at 24 hours poststroke (acute group) and 9 patients received the first dose on day 7 poststroke (subacute group; n = 3 at each dose in each group). A scheduled administration of G-CSF was skipped if the patient's leukocyte count exceeded 40,000/μL. Patients received neurologic and MRI examinations. *Results:* We found neither serious adverse event, drug-related platelet reduction nor splenomegaly. Leukocyte levels remained below 40,000/μL at 150 and 300 μg G-CSF/body/day, but rose above 40,000/μL at 450 μg G-CSF/body/day. Neurologic function improvement between baseline and day 90 was more marked after treatment in the acute phase versus the subacute phase (Barthel index 49.4 ± 28.1 *v* 15.0 ± 22.0; $P < .01$). *Conclusions:* Low-dose G-CSF (150 and 300 μg/body/day) was safe and well tolerated in ischemic stroke patients, and leukocyte levels remained below 40,000/μL. **Key Words:** Acute stroke—granulocyte-colony stimulating factor—ischemic stroke—stroke trials.

Research on stem and progenitor cells has the potential to yield new treatments for ischemic stroke, but transplantation of these cells faces a variety of problems, such as infection, rejection, and risk of malignancy, and there are also ethical and political issues.[1] Granulocyte colony-stimulating factor (G-CSF; filgrastim), which is in widespread clinical use for the treatment of chemotherapy-associated neutropenia,[2] is a new candidate for neuroprotection and neuroregeneration. Because its profiles of pharmacologic and adverse effects are well known, the clinical application of G-CSF is expected to be

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straightforward when compared with the application of stem/progenitor cell therapy.

As described in our forthcoming review article,[3] G-CSF may be useful for treatment of acute ischemic stroke because of its anti-inflammatory, antiapoptotic, and neurogenesis-promoting properties. Several clinical studies using either subcutaneous[4-8] or intravenous[9] G-CSF injections in patients with acute cerebral infarction have been reported. However, maximum leukocyte counts in those studies exceeded 40,000/μL,[4,6,7,9] and it is well known that increased levels of peripheral neutrophils may result in aggregate formation in the cerebral microvasculature, leading to a breakdown of blood flow and worsening brain damage.[10] A negative effect of G-CSF on outcome, associated with enhanced brain atrophy and an exaggerated inflammatory response, has also been reported in permanent cerebral infarction.[11] We decided to use intravenous administration of G-CSF, but not subcutaneous administration, because Higashi et al[12] reported that maximum leukocyte counts were lower after the intravenous administration of G-CSF than after subcutaneous administration in normal volunteers. Therefore, if intravenous administration of low-dose G-CSF can improve clinical outcomes without inducing adverse effects, it may be appropriate for the treatment of ischemic stroke. We examined the safety and tolerability of low-dose intravenous G-CSF at lower doses (150, 300, and 450 μg/body/day for 5 consecutive days) than have been used in previous reports.[4,6-9]

We have already established that the administration of hematopoietic cytokines in the subacute phase of cerebral infarction is effective for functional recovery, facilitating the proliferation of intrinsic neural stem/progenitor cells and the transition of bone marrow–derived neuronal cells[13] and providing a favorable microenvironment for neurogenesis through the upregulation of interleukin-10 in mice.[14] On the other hand, the administration of G-CSF in the acute phase of cerebral ischemia is expected to induce various neuroprotective mechanisms, including inhibition of glutamate release,[15] reduction of inflammation,[16,17] antiapoptosis activity,[18] and suppression of edema formation.[19] Therefore, we investigated the effect of low-dose G-CSF given intravenously in the acute phase (at 24 hours) or subacute phase (at day 7) of ischemic stroke on clinical outcome and magnetic resonance imaging (MRI) findings at day 90 after onset.

Methods

Study Design

We performed a prospective phase I study of G-CSF in patients with acute or subacute ischemic stroke. This is the first such clinical study in Japan, and it was approved by the Ethics Committee of Tokai University Hospital (July 26, 2007; no. 06R-093).

We recruited patients with a first episode of MRI-confirmed acute cerebral infarction localized in the territory of the middle cerebral artery (MCA) who presented to our department at Tokai University Hospital within 24 hours after the onset of stroke caused by atherothrombosis or cardioembolism. Patients were eligible if they were between 45 and 79 years of age and had a score of between 4 and 22 on the National Institutes of Health Stroke Scale (NIHSS). We excluded patients with other intracranial pathologies (e.g., tumors or infection), a history of major bleeding requiring blood transfusion, leukocytosis (>15,000/mm^3), thrombocytopenia (<140,000/mm^3), hepatic dysfunction (>100 IU/L in aspartate aminotransferase or alanine aminotransferase), renal dysfunction (>1.5 mg/dL in creatinine), evidence of malignant disease or congestive heart failure, or splenomegaly (>11 cm in length or >5 cm in width on ultrasonography).[20]

Written informed consent was obtained from each patient. If the patient was unable to consent (e.g., because of confusion or dysphasia), proxy consent was obtained from a relative. Patients were assigned to acute (first dose of G-CSF within 24 hours of onset [n = 9]) and subacute (first dose of G-CSF at day 7 after onset [n = 9]) groups. Within each group, patients were assigned to 1 of 3 intravenous dose regimens according to the dose escalation plan (150, 300, and 450 μg/body/day for 5 consecutive days [n = 3 for each dose]). The trial used the standard 3 + 3 dose escalation design. The study drug dose was escalated to the next higher level if none of the first 3 patients developed treatment-related adverse events (TAEs), such as thrombocytopenia, splenomegaly, liver and renal dysfunctions, etc. If 1 of the first 3 patients developed a TAE, up to 3 additional patients were to be enrolled for treatment at the same dose level. If no further TAEs were encountered, dose escalation resumed. The safety committee monitored the response of each group of 3 patients, and its approval was required before treatment of the next group of 3 patients was commenced. Each daily dose was administered in 2 equal parts at noon and midnight, starting at noon on the first day.

We monitored the leukocyte counts of all patients every 6 hours for 6 days after the start of G-CSF administration. A scheduled administration of G-CSF was skipped if the patient's leukocyte count exceeded 40,000/μL. Subsequent scheduled G-CSF administrations were restarted when the leukocyte count fell below 30,000/mm^3. It was decided in advance that if a patient's leukocyte count reached 70,000/μL, we would perform leukapheresis with a Cobe Spectra cell separator (Terumo BCT Co, Lakewood, CO). We also monitored spleen size by means of ultrasonography 3 times before G-CSF therapy and at days 3 and 5 after the start of G-CSF therapy. It was also decided in advance that if the spleen size became enlarged to >13 cm in length and >6 cm in width according to the ultrasonographic assessment, the administration of G-CSF would be stopped to avoid rupture of the spleen.[21]