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Brain Research 1041 (2005) 125–131
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Granulocyte colony-stimulating factor induces sensorimotor recovery in intracerebral hemorrhage

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Accepted 16 November 2004
Available online 23 March 2005

Abstract

Granulocyte colony-stimulating factor (G-CSF) has been used in the treatment of neutropenia in hematologic disorders. The neuroprotective and anti-inflammatory effects of G-CSF were reported in various neurological disease models. In this study, we examined whether G-CSF induces functional recovery after intracerebral hemorrhage (ICH). ICH was induced using collagenase injection in adult rats. Either G-CSF (50 μg/kg, i.p.) or saline was given from 2 h after ICH and every 24 h for 3 days. 72 h after ICH induction, the rats were sacrificed for histological analysis and measurement of brain edema. Behavioral tests were performed before and 1, 7, 14, 21, 28, and 35 days after ICH. We also measured the blood–brain barrier (BBB) permeability using Evans blue dye injection method. G-CSF-treated rats recovered better on rotarod and limb placing tests, starting from 14 days throughout 5 weeks after ICH. The brain water content and BBB permeability of G-CSF-treated group decreased in the lesioned hemispheres compared with those of ICH-only group. In G-CSF-treated group, the number of TUNEL$^+$, myeloperoxidase$^+$, and OX42$^+$ cells was smaller than that of ICH-only group in the periphery of hematoma. These findings suggest that G-CSF induces long-term sensorimotor recovery after ICH with reduction of brain edema, inflammation, and perihematomal cell death.

Theme: Disorders of nervous system
Topic: Neurotrophic factors: biological effects

Keywords: G-CSF; Intracerebral hemorrhage; Brain edema; Inflammation; Atrophy

1. Introduction

Intracerebral hemorrhage (ICH) represents at least 10–15% of all strokes in the Western population [20]. Medical therapy for patients with ICH is limited to only supportive care or invasive evacuation of hematoma in selective patients. Previous studies on ICH indicated that brain injury can be caused by inflammatory mediators released from the blood and leukocytes [2,8,13,18,19], characterized by the induction of parenchymal inflammation within hours of ICH occurrence. It is initiated by adherence of leukocytes to damaged brain endothelia and subsequent brain entry [19]. TNF-α, matrix metalloproteinases (MMPs; especially

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doi:10.1016/j.brainres.2004.11.067

MMP-12), adhesion molecules, and glutamate levels have been reported to be up-regulated after ICH [8,19].

Granulocyte colony-stimulating factor (G-CSF), a 20-kDa protein, is a member of the cytokine family of growth factors. It stimulates the proliferation, survival, and maturation of the cells committed to the neutrophilic granulocyte lineage [10]. G-CSF expands the monocyte/macrophage subset and promotes an anti-inflammatory pattern conferring protection in murine endotoxemia [9]. Moreover, T cell allogenic and mitogenic reactivities are inhibited in G-CSF-treated individuals [15], corresponding to a reduced interferon-γ production capacity [11]. It is known that G-CSF exerts anti-inflammatory and pro-Th_2 effects via JAK-STAT signaling to trigger an imbalance in the cytokines produced by lymphocytes, resulting in the reduction of interferon-γ and TNF-α release and increase in the levels of antagonists for inflammatory cytokines [2,6,9,27]. This could reduce cytokine toxicity, neutrophil activation, and infiltration [12]. The anti-inflammatory effect of G-CSF provided durable protection from experimental autoimmune encephalomyelitis (EAE) [27]. G-CSF administration modulates and enhances endogenous bone marrow stem cells to acquire neuronal characteristics [5] and have anti-apoptotic and neuroprotective effects on neuronal cells [22].

In the present study, we examined the two hypotheses. First, G-CSF treatment may have anti-inflammatory action in the setting of ICH. Second, G-CSF treatment may also reduce the brain edema and perihematomal cell death following ICH and improve functional outcome.

2. Materials and methods

2.1. Induction of intracerebral hemorrhage and G-CSF treatment

All the procedures were performed following an institutionally approved in accordance with NIH *Guide for the Care and Use of Laboratory Animals*. 163 male Sprague–Dawley rats (Daehan Bio, Seoul, R.O.K.) weighing 200 to 220 g were used in these experiments. Rats were grouped as ICH-only group (n = 72), G-CSF-treated ICH group (n = 78), sham-operated group (n = 9), and G-CSF-treated normal group (n = 4). Experimental ICH was induced by the stereotaxic intrastriatal administration of bacterial collagenase type IV (Sigma), as described elsewhere [4,12,13,21]. In brief, after intraperitoneal injections of 1% ketamine (30 mg/kg; Sigma) and xylene hydrochloride (4 mg/kg; Sigma), rats were placed in a stereotaxic frame (David Kopf instruments, Tujunga, CA), and a burr hole was made. 30-gauge Hamilton syringe needle was inserted into the striatum. ICH was induced by the administration of 1 μl containing 0.23 CDU of collagenase type IV (Sigma) over 5 min. After completion of collagenase infusion, craniotomies were sealed with bone wax, and rats were allowed to recover.

The recombinant human G-CSF (50 μg/kg, Kirin, Japan), dissolved in 2 ml of 0.9% saline, was administered intraperitoneally, 2 h after ICH induction and daily afterward for 3 days. Saline was also given to the ICH-only group during the same period. Physiologic parameters, including mean arterial blood pressure, blood gases, and glucose concentration, were measured during the experiment.

2.2. Behavioral testing

Behavioral testing (n = 15, respectively) was performed weekly using the rotarod and modified limb placing tests (MLPT), which were monitored by two individuals blinded to rat treatment status. In the rotarod test [4,12], the rats were placed on the accelerating rotarod cylinder, and the time the animals remained on the rotarod was measured. The animals were trained for 3 days before stereotaxic operation. The maximum duration (in seconds) on the device was recorded with 3 rotarod measurements 1 day before ICH induction. Motor test data are presented as percentages of the maximal duration compared with the baseline control (before ICH). The MLPT is a modified version of a test previously described [4,12,13]. The test consists of two limb-placing tasks that assess the sensorimotor integration of the forelimb and the hindlimb by checking responses to tactile and proprioceptive stimulation. First, the rat is suspended 10 cm over a table and the stretch of the forelimbs towards the table is observed and evaluated: normal stretch, 0 points; abnormal flexion, 1 point. Next, the rat is positioned along the edge of the table, with its forelimbs suspended over the edge and allowed to move freely. Each forelimb (forelimb—second task, hindlimb—third task) is gently pulled down and retrieval and placement are checked. Finally, the rat is placed towards the table edge to check for lateral placement of the forelimb. The three tasks are scored in the following manner: normal performance, 0 points; performance with a delay (2 s) and/or incomplete, 1 point; no performance, 2 points. 7 points means maximal neurological deficit and 0 points means normal performance.

2.3. Measuring brain water contents and BBB permeability

Three days after operation, rats were anesthetized and sacrificed by decapitation (n = 12 respectively). The brains were removed immediately and divided into two hemispheres along the midline then the cerebellum was removed. The brain samples were immediately weighed on an electronic analytical balance to obtain the wet weight and then dried in a gravity oven at 100 °C for 24 h to obtain the dry weight. Water contents were expressed as a percentage of wet weight [4,13].

To evaluate vascular permeability, a quantitative fluorescent detection of extra-vasated Evans blue dye was used as described elsewhere [2]. Briefly, Evans blue (3 ml/kg of 2% in 0.9% normal saline) was injected into