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Granulocyte colony stimulating factor (GCSF) improves memory and neurobehavior in an amyloid-β induced experimental model of Alzheimer's disease

Ajay Prakash [a], Bikash Medhi [b,*], Kanwaljit Chopra [a]

[a] Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India
[b] Department of Pharmacology, Postgraduate Institute of Medical Education & Research, Chandigarh 160012, India

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ABSTRACT

GCSF is an endogenous neuronal hematopoietic factor that displays robust *in vitro* and *in vivo* neuroprotective activity. The present study aimed to evaluate the effect of GCSF on Aβ-induced memory loss in an Alzheimer's disease model of rats. A total of 42 male adult Wistar rats weighing 200–250 g were used in the study and were divided into 7 experimental groups. Animals were subjected to intracerebroventricular (ICV) injection stereotaxically at day 0 to instill amyloid-β_{1-42} ($A\beta_{1-42}$) or PBS (sham operated group) at 10 μl (5 μl bilaterally). GCSF treatment was given from day 7 to 12 of Aβ injection. On day 21, behavioral tests (short term memory, exploratory behavior and motor coordination) in all groups were evaluated. Biochemical parameters and RNA expression were measured to ensure the efficacy of GCSF. GCSF (35 and 70 μg/kg, s.c.) showed statistically significant improvement in memory as compared to control and sham operated groups ($p < 0.05$). Mean time spent in the platform placed quadrant was found to be significantly increased in the GCSF (70 μg/kg, s.c.) as compared to GCSF (35 μg/kg, s.c.) and GCSF (10 μg/kg, s.c.) groups ($p < 0.001$). GCSF (35 and 70 μg/kg, s.c.) also improved motor coordination and exploratory behavior significantly as compared to naïve sham operated and GCSF (10 μg/kg, s.c.) groups ($p < 0.05$). Improvement in memory by GCSF (35 and 70 μg/kg, s.c.) was coupled with marked reduction of lipid peroxidation, acetylcholinesterase levels and a significant increase in antioxidant enzymes as well as total RNA expression in the brain. Additionally, GCSF (35 and 70 μg/kg, s.c.) significantly increased progenitor cells (iPSCs) and surface marker CD34+ in the brain and hence induced neurogenesis. The present findings demonstrate an improvement of memory and neurobehavioral function with GCSF in Aβ-induced Alzheimer's disease model in rats.

1. Introduction

Alzheimer's disease (AD) is a progressive, chronic neurodegenerative disease, which is considered as the 5th leading cause of death for those older than the age of 65 and 7th leading cause of death in the United States of America (Jacobsen et al., 2005; Zhao et al., 2008). An epidemiological study reveals that the number of Alzheimer cases is expected to double every 20 years; however, overall dementia was found in more than 35 million people worldwide (Querfurth and LaFerla, 2010). Experimental studies affirmed that the degeneration in the hippocampus and dentate gyrus (DG) leads to progressive loss of cognition and learning (Salloway et al., 2008; Fernández-Verdecia et al., 2009).

Anticholinesterases and N-methyl-D-aspartate (NMDA) antagonists are the major treatment modalities available for symptomatic relief. Replacement therapy with stem cells and growth factors is the proposed latest strategy for the management of Alzheimer's disease (Salloway et al., 2008; Hou and Hong, 2008; Fernández-Verdecia et al., 2009). Growth factors such as erythropoietin (EPO) (Solaroglu et al., 2003; Assaraf et al., 2007), granulocyte colony stimulating factor (GCSF) (Park et al., 2005; Sanchez-Ramos et al., 2009), and brain-derived neurotrophic factors (BDNFs) (Schabitz et al., 2000; Angelucci et al., 2010) come second to stem cells as new interventions in neurological disorders and demonstrate their efficacy by inducing the progenitor stem cells in the circulation. These have been tried in neurotrauma (Kulbatski et al., 2005), stroke (Schabitz et al., 2000; Gibson et al., 2005; Sprigg et al., 2006), Alzheimer's disease (Rowe et al., 2009; Sanchez-Ramos et al., 2009; Querfurth and LaFerla, 2010) and Parkinson's Disease (Zhao et al., 2008; Hou and Hong, 2008).

Experimental evidence showed accumulation of Aβ as a primary pathological change in the development of Alzheimer's disease (AD).

Abbreviations: GCSF, granulocyte colony-stimulating factor; ICV, intracerebroventricular; AD, Alzheimer's disease; $A\beta_{1-42}$, amyloid-beta (1–42); DG, dentate gyrus; NMDA, N-methyl-D-aspartate; EPO, erythropoietin; BDNF, brain-derived neurotrophic factors; NBT, nitro blue tetrazolium; EDTA, ethylenediamine tetrachloroacetic acid; TBA, thiobarbituric acid; TLT, transfer latency time; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; nAChR, nicotinic receptor; mAChR, muscarinic receptor.

* Corresponding author at: Department of Pharmacology, PGIMER, Chandigarh, India. Tel.: +91 1722755250 (office), +91 9815409652 (mobile); fax: +91 1722744043.
E-mail address: drbikashus@yahoo.com (B. Medhi).

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Cleary et al. (2005) provided first experimental evidence that a defined molecular species of the Aβ protein interferes with cognitive function and showed that soluble trimers and dimers Aβ were necessary and sufficient to disrupt learned behavior. In addition, recently it was shown that soluble Aβ disrupted the memory of a learned behavior in normal rats and that the mechanism involved metabotropic glutamate receptors and N-methyl-D-aspartate (NMDA) receptors (Shankar et al., 2008).

GCSF is a multi-modal hematopoietic growth factor which has been approved by the US-FDA to decrease the incidence of infection manifested by febrile neutropenia in patients receiving myelosuppressive anticancer drugs. Sanchez-Ramos et al. (2009) demonstrated that the GCSF significantly decreased brain amyloid β and reversed cognitive impairment in Alzheimer's mice. Experimental studies have demonstrated that GCSF has the neuroprotective activity in stroke and neurotrauma (Kleinschnitz et al., 2004; Kulbatski et al., 2005; Gibson et al., 2005a). Sprigg et al. (2006) showed that GCSF effectively mobilized bone marrow CD34+ stem cells in patients with recent ischemic stroke and proved it to be a neuroprotective agent.

Hence, based on the previous studies, the present study was designed to evaluate GCSF in improving the memory and neurobehavioral deficit in Alzheimer's disease in rats. Moreover, an attempt was made to delineate its mechanism of action.

2. Materials and methods

2.1. Animals

A total of 42 adult Wistar male rats weighing 200–250 g were obtained from the Central Animal House of the Institute. The animals were housed in standard laboratory conditions at 25 ± 2 °C, humidity of 60 ± 2% and 12 h light:dark cycle. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University and performed in accordance with the guidelines of the Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. The animals had free access to standard laboratory rat chow diet and tap water. The animals were acclimatized to the laboratory conditions 1 week prior to experimentation. All experiments were conducted daily between 09:00 am and 03:00 pm.

2.2. Drugs and chemicals

The amyloid-β_{1-42} (Aβ) was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA) to induce Alzheimer's disease in the rats. GCSF (filgrastim), marketed product of Dr. Reddy's Laboratories Ltd. was directly purchased from the market. RNA extraction kit was obtained from Real Biotech Corporation, Taiwan.

3. Methods

Experiment was carried out in a parallel design method and the rats were divided into the following six groups; *Control group* (n = 6): Group represented, healthy normal rats which were treated with the vehicle (PBS, pH 7.4) subcutaneous (s.c.) from 7 to 12 days and assessed on days 0, 7, 14, and 21; *Control (GCSF) group* (n = 6): Comprised of healthy normal rats treated with GCSF 70 μg/kg, s.c. from 7 to 12 days and assessed on days 0, 7, 14, and 21. *Sham operated group* (n = 6): Rats were exposed to stereotactic/intracerebroventricular PBS injection and assessed on days 0, 7, 14, and 21; *Dementia (Aβ) group* (n = 6): Rats were exposed to stereotaxic surgery and Aβ(1–42) was injected in a volume of 10 μl of Aβ(1–42) aggregate (5 μl bilateral) in PBS, pH 7.4 and allowed to develop the symptoms of AD for 7 days and assessed on days 0, 7, 14, and 21; *GCSF 10 group*: After administration of Aβ(1–42)-aggregates, rats were treated with GCSF (10 μg/kg, s.c.) from 7 to 12 days and assessed on days 0, 7, 14, and 21; *GCSF 35 group*: After administration of Aβ(1–42)-aggregates, rats were treated with GCSF (35 μg/kg, s.c.) from 7 to 12 days and assessed on days 0, 7, 14, and 21, and *GCSF 70 group*: After administration of Aβ-aggregates, rats were treated with GCSF (70 μg/kg, s.c.) from 7 to 12 days and assessed on days 0, 7, 14, and 21. On day 22, the neurobehavioral parameters, brain biochemical assessment, and GCSF-induced RNA expression were assessed. Immunohistopathological study was performed to study the pathological changes in lateral ventricles and hippocampus region of rat brain. Brief experimental design is explained in Fig. 1.

3.1. Alzheimer's disease model development

The Aβ aggregate was prepared from a solution of amyloid-β_{1-42} (Sigma-Aldrich Inc.) in PBS, pH 7.4. The solution was incubated at 37 °C for 3 days to form the aggregated Aβ and stored at −70 °C. Animals were anesthetized with 40 mg/kg, i.p. sodium pentobarbital, and the injection of aggregated Aβ was made bilaterally into lateral ventricle using a 27-gauge needle connected to a micro syringe (Hamilton) with the help of stereotactic apparatus at coordinates: AP: 0.8 mm to bregma; lateral: 1.5 mm to sagittal suture and 3.6 mm beneath the surface of the brain (Sharma and Gupta, 2002). Total volume of ICV injection was 10 μl of aggregated Aβ or PBS bilaterally (5 μl/burr hole) and thereafter, the rats were housed and observed for 7 days for AD symptoms to develop (Stephan et al., 2001; Yan et al., 2001).

3.2. Learning and memory behavioral study

3.2.1. Morris water maze test

The rats were tested for memory in a spatial version of Morris water maze test (Morris et al., 1982; Tuzcu and Baydas, 2006) using computer tracking system with EthoVision software (Noldus Information Technology, Wageningen, Netherlands). The apparatus consisted of a circular water tank (180 cm in diameter and 60 cm high). A platform (12.5 cm in diameter and 38 cm high) invisible to the rats, was set 2 cm below the water level inside the tank with water maintained at 25.5 ± 2 °C at a height of 40 cm. The tank was located in a large room where there were several brightly colored cues external to the maze; these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the study. The rats received four consecutive daily training trials in the following 5 days, with each trial having a ceiling time of 90 s and a trial interval of approximately 30 s. For each trial, each animal was put into the water at one of four starting positions, the sequence of which being selected randomly. During test trials, the rats were placed into the water tank at the same starting point, with their heads facing the wall. The animal had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 20 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the animal failed to reach the escape platform within the maximally allowed time of 90 s, it was guided with the help of a rod and allowed to remain on the platform for 20 s. The time to reach the platform (escape latency in seconds) and total distance traveled to reach the platform (path length in cm) was measured as parameter of memory assessment.

3.2.2. Memory consolidation test

Test was performed to observe the consolidation process of memory described earlier by Tuzcu and Baydas (2006). This is based on the principle that the total time spent in the target quadrant (quadrant in which platform was hided) indicates the degree of memory consolidation that has taken place after learning. Briefly, the rats were placed into the water maze as in the training, except that the hidden platform was removed from targeted quadrant and the time spent for crossing targeted quadrant was recorded for 90 s.

3.2.3. Short-term memory evaluation using elevated plus maze

Plus maze consisted of 2 open (16 × 5 cm) and two enclosed (16 × 5 × 12 cm) arms, connected by a central platform. The apparatus