

Effect of filgrastim (recombinant human granulocyte colony stimulating factor) on spatial memory in aged rats

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ABSTRACT


Background: Apart from functioning as a multimodal hematopoietic growth factor, granulocyte colony-stimulating factor (G-CSF) causes intense consequences on the brain. It has been viewed that G-CSF boosts the improvement from the neurologic deficits in rodent models of central nervous system diseases. **Aims and Objective:** To evaluate the efficacy of G-CSF as an intervention for improving cognitive deficits commonly associated with aging. **Materials and Methods:** In this study, male Wistar rats aged 21 months were treated for 2 weeks with G-CSF intraperitoneally at doses of 10, 50, and 70 mg/kg/day. The learning process was assessed by the reference memory task in the Morris water maze, by comparing the G-CSF-treated group with the control animals. All the rats received Morris water maze training (four trials/day for 5 days) in order to assess the hippocampal-dependent spatial learning and received a 60-s probe trial test of spatial memory retention 24 h after the twentieth trial. **Result:** Over 5 days of training, G-CSF (10, 50, and 70 mg/kg/day) significantly reduced the latency and path length to finding the escape platform ($P < 0.01$). In probe trials (without platform), on the last day of training, the G-CSF-treated group spent significantly longer time in the platform quadrant when compared with the control animals ($P < 0.01$). Among the treated groups, the 50-mg/kg dosage of G-CSF induced the best rehearsals memory. **Conclusion:** The findings observed in this study support G-CSF as a promising therapeutic aid for cognitive enhancement in the aging phenomenon.

KEY WORDS: Memory; G-CSF; Filgrastim; Morris Water Maze; Aging

INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) is a member of the cytokine family of growth factors. It acts as a potent hematopoietic factor that enhances the survival and drives differentiation of myeloid lineage cells, resulting in the generation of neutrophilic granulocytes.^[1] The endothelium,

macrophages, and several other immune cells are involved in the formation of G-CSF. G-CSF and its receptor have also been reported to be expressed in neurons and neural progenitor cells that reside in the hippocampal neurogenic niche.^[2] G-CSF passes through the intact blood-brain barrier and possesses putative neuroprotective properties thought to be related to its anti-inflammatory effects and the ability to block neuronal programmed cell death through the interactions with G-CSF receptors.^[3,4] G-CSF may also be a potent neuronal growth factor. These properties make G-CSF a promising candidate for treating cognitive impairment in the elderly persons. Cognitive impairment and dementia are the common serious health problems, which impair the quality of life in the elderly people. It has been reported by various studies that, as the age of humans increases, there is a steady decrease in the features such as learning and memory.^[5,6] Furthermore, owing to this

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deterioration associated with the aging process, deficits in the spatial learning and memory tasks occur.^[7-11] These age-related spatial learning and memory deficits have been attributed, in part, to the alterations in the connections and function of hippocampal formation.^[12,13] A previous study indicated that G-CSF administration can reverse the cognitive impairment in a commonly used Alzheimer disease mouse model.^[14] Accordingly, the G-CSF treatment improved the spatial learning and reacquisition of information in rats engaged in a radial maze, presumably, by promoting the survival of new neurons^[3]; in a murine model of pneumococcal meningitis, G-CSF increased the hippocampal neurogenesis and improved the spatial learning performance. For the cognitive benefits of G-CSF, multiple mechanisms, including reduction of inflammation, stimulation of neurogenesis and synaptogenesis, activation of resident microglia, and the induction of phagocytosis have been proposed.^[14,15] However, G-CSF-driven memory recovery in the elderly persons remains underexplored. This study investigated the effects of G-CSF treatment in aged rats by using behavioral parameters of cognitive performance in Morris water maze (MWM).

MATERIALS AND METHODS

Animals

Twenty-four-month-old rats weighing 400–450 g were obtained from the Aging Farm, which was established at our animal house and used as the aged rats. The aged rats were produced by keeping them for 21 months in the Aging Farm after purchase from the same breeder at an age of 3 months. They were housed two per cage in a temperature ($23 \pm 1^\circ\text{C}$) and light (12-h light/dark schedule; lights on at 8 a.m.)-controlled environment and fed laboratory food and water ad libitum. All the protocols for the experiments on animals were approved by the Research and Ethics Committee of Golestan University of Medical Sciences, Golestan, Iran. The animals were randomly divided into four groups of eight animals each, including control (C) (untreated) group, and 10-, 50-, and 70-mg/kg filgrastim groups. Filgrastim was purchased from Ariatinagen Company (Gorgan, Iran), and after dissolving in saline, administered intraperitoneally at doses of 10, 50, and 70 mg/kg to the rats for 2 weeks. In the last week, the injections were administered 60 min before the MWM task.

MWM Task

A black circular pool (160 cm diameter, 60 cm high) filled with water (30 cm depth) at $24 \pm 2^\circ\text{C}$ was utilized as the MWM for the study. Four quadrants arbitrarily designed as northeast (NE), northwest (NW), southeast (SE), and southwest (SW) areas were segmented in the pool. One centimeter below the water surface was concealed a submerged Plexiglas platform (10 cm \times 10 cm) and placed in a particular place in the center of the NW quadrant. The animals underwent 5 days of training with the hidden platform; four training sessions with a 60-s

intersession interval were performed on each day. A rat was kept with its face toward the wall of the pool at any one of the three start points and the trial initiated. The start location was varied on each training trial and changed each day. The trial was terminated when the animal entered the platform. If the rat did not find the platform within 60 s, it was placed on the platform by the experimenter for 15 s. During the acquisition of the spatial navigation task, all the groups were given one session of four trials each day (days 1–5; trials 1–20). Spatial memory was evaluated in the probe trial. On the sixth day (trial 21), the platform was removed, and the animals were allowed to swim for 60 s. By using a computerized video tracking system (Maze router, Urmia Instruments, Inc.), the path of the animals in the maze was observed. The parameters such as the time taken to reach the platform (latency), swimming speed, and swim path length (SPL) were determined in the training trials. Moreover, during the probe trials, an estimation of the percentage run time of each experimental animal within the quadrant of the water bath where the hidden platform had been placed in the training trials was done.

Statistical Analysis

Data were analyzed using SPSS software, version 11.5 and plotted as mean \pm SD. The comparison among the groups was made using analysis of variance (ANOVA) with a post hoc Tukey test or repeated-measures ANOVA. The results were considered significant when $P < 0.05$ and highly significant when $P < 0.01$.

RESULTS

Acquisition

The escape latency and the distance traveled by the rats to finding the hidden platform in the water maze task are shown in Figures 1A and 1B, respectively. Animals from all the groups progressively improved their ability to find the platform over the 5 days of acquisition ($P < 0.05$). However, repeated-measures ANOVA of these data revealed that the performance on this task differed between the control and filgrastim-treated groups in the trial days ($P < 0.01$). Inspection of the data in these days showed that animals in filgrastim-treated groups learned the task at a high rate, traveled a shorter distance, and spent less time to find the escape platform than the control group ($P < 0.01$), but there was no significant difference observed in these data among the groups that received filgrastim on each trial day. The analysis of swimming speed by using two-way ANOVA also showed no significant differences as the training days progressed among the groups and no interaction between the days and the groups [Figure 2].

Probe Trial

By applying a different starting position, a 60-s free-swim probe trial was carried out, and the retention of the spatial training was assessed 24 h after the last training session. The parameters such as the time spent in the quadrant containing

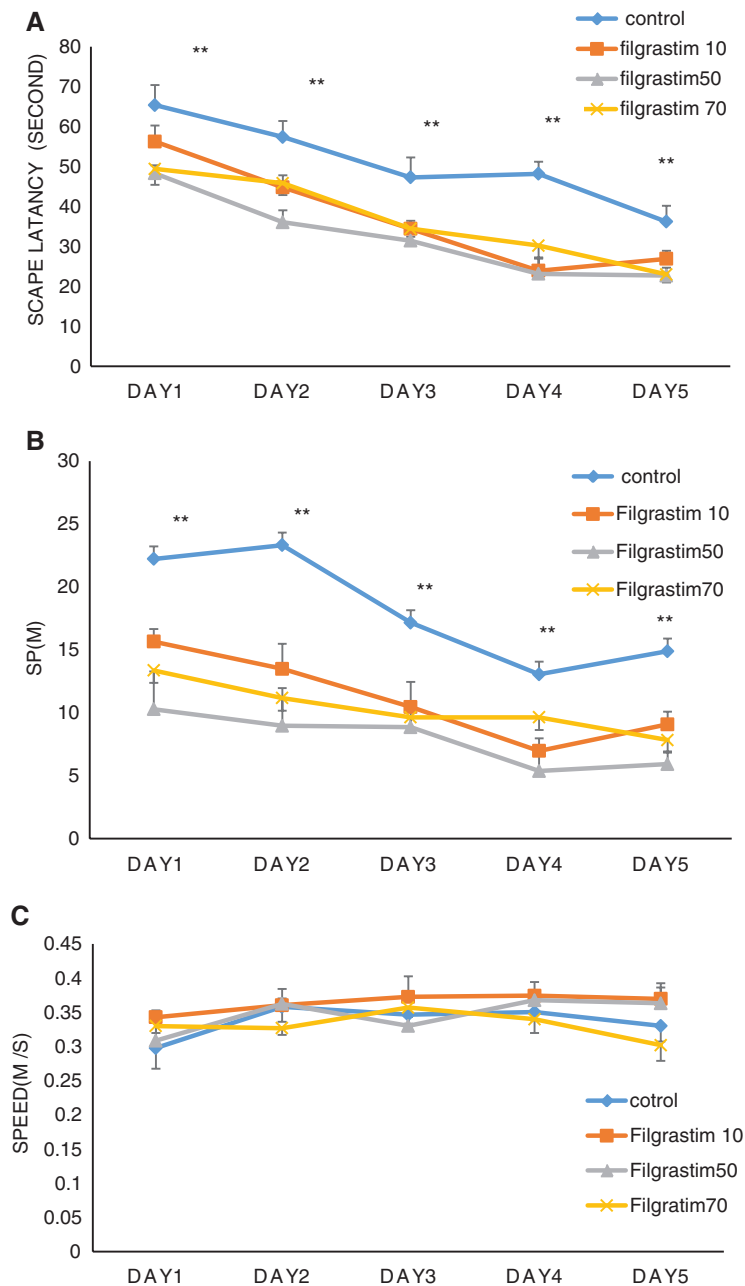


Figure 1: Effects of short-time treatment with filgrastim (14 days) on the performance of spatial memory acquisition phase in MWM. Average escape latency (A), distance traveled (B), and swimming speed (C) within each day (made up of four trials) are shown. Asterisks indicate a significant difference from the control group (** $P < 0.01$).

the platform during the training (target quadrant) were determined from the probe trial. Post hoc analysis showed that the filgrastim-treated group was significantly different from the control ($P < 0.01$) group. The filgrastim-treated animals spent more time in the training quadrant than the control animals in the 60-s probe trial [Figure 2]. In addition, among the treated groups the 50-mg/kg/day group was found to have spent more

time in the training quadrant ($P < 0.01$). Figure 3 shows the representative plots (top view) of individual swim paths of one rat for each treatment group and probe trial. In these examples, the start location was in the NW quadrant of the pool, and the platform had formerly been located in the NE quadrant of the pool (indicated by small circle for illustration purposes only; note that no platform was present in probe trials).

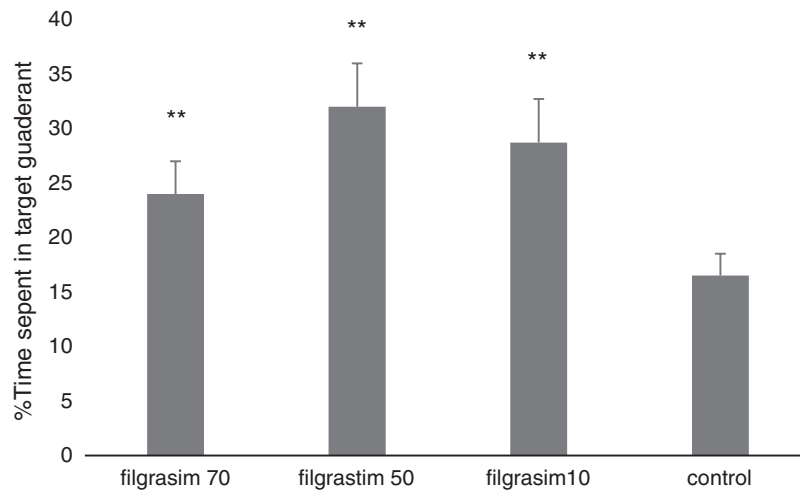


Figure 2: Mean (± SEM) percentage of the time spent in target quadrant in MWM during the probe trials. Asterisks indicate a significant difference from the control group (** $P < 0.01$).

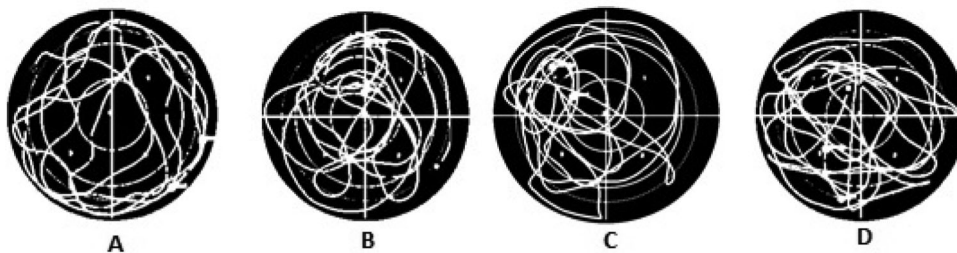


Figure 3: Tracings of the typical swim patterns in the probe trials (trial 21) at the control (A), and 10- (B), 50- (C), and 70-mg/kg (D) filgrastim groups tested. The escape platform was on the NW quadrant.

DISCUSSION

There are now several lines of evidence suggesting that G-CSF possesses neuroprotective and anti-inflammatory effects in various neurological disease models and can improve cognition and memory. In this study, we examined the effects of G-CSF on spatial memory in aged rats. The animals were treated for 2 weeks with different doses of filgrastim, and their performance in a reference memory task—the water maze, was compared with the vehicle-treated controls. The rats treated with 10, 50, and 70 mg/kg/day of filgrastim performed significantly better than the control group in the water maze task. Latency and path length to finding the escape platform significantly reduced in the filgrastim-treated animals. In a probe trial, where the platform was removed from the pool, the filgrastim-treated groups were also shown to spend significantly longer time in the platform quadrant than control animals, demonstrating a better ability of remembering the position of the platform. Among the filgrastim-treated groups, the 50-mg/kg dosage of filgrastim induced the best rehearsals. In a similar study, Sikoglu et al.^[16] observed the immediate effects of G-CSF in spatial memory recovery in a rat model of traumatic brain

injury. Our results also showed an immediate and beneficial effect of filgrastim on cognitive function. The mechanism of the G-CSF memory enhancing is unknown. Previous studies on intracerebral hemorrhage indicated that the beneficial effect of G-CSF on brain might be mediated through its anti-inflammatory effect.^[17] In addition, it is possible that G-CSF memory enhancer effect was not global but impactful in the brain regions involved in spatial learning.^[18] G-CSF has been known to exhibit antiapoptotic effects in mature neurons and activate multiple cell survival pathways.^[19] Both the G-CSF and its receptors are widely expressed by neurons in the central nervous system,^[20] and G-CSF can activate the transcription factor cAMP response element-binding protein (CREB) through the mitogen-activated protein kinase pathway. The CREB plays an important role for long-term potentiation, a cellular process associated with learning and memory.^[21] Accordingly, G-CSF treatment improved spatial learning and reacquisition of information in rats engaged in a radial maze, presumably, by promoting the survival of new neurons,^[22] which suggests an autocrine protective signaling mechanism that can improve memory in elderly people. We, therefore, propose G-CSF as a potential new drug for enhancing memory in the elderly people.

CONCLUSION

Daily IP injections of filgrastim at doses of 10, 50, and 70 mg/kg for 2 weeks improved aged rats' performance in the MWM, and, hence, although a low dose of 10 mg/kg can exert significant memory enhancer effect in old rats, a daily injection of 50 mg/kg filgrastim showed a good spatial memory enhancing effect in the aged rats.

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