Neuroscience Letters 564 (2014) 16–20

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Granulocyte colony-stimulating factor fails to enhance leptomeningeal collateral growth in spontaneously hypertensive rats

Yukio Sugiyama, Yoshiki Yagita*, Toshiro Yukami, Akihiro Watanabe, Naoki Oyama, Yasukazu Terasaki, Emi Omura-Matsuoka, Tsutomu Sasaki, Hideki Mochizuki, Kazuo Kitagawa

Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan

HIGHLIGHTS

- G-CSF promoted the growth of leptomeningeal anastomoses in normotensive rats.
- Hypertension impaired hypoperfusion-induced collateral growth.
- G-CSF could not restore the collateral growth in SHR.
- G-CSF-mediated tissue macrophage mobilization was not observed in SHR.

ARTICLE INFO

Article history:
Received 21 November 2013
Received in revised form 8 January 2014
Accepted 25 January 2014

Keywords:
Arteriogenesis
Granulocyte colony-stimulating factor
Brain hypoperfusion

ABSTRACT

The promotion of collateral artery growth is an attractive approach for the treatment of chronic brain hypoperfusion due to occlusive artery disease. We previously reported that hypertension impaired the collateral artery growth of leptomeningeal anastomoses after brain hypoperfusion. Granulocyte colony-stimulating factor (G-CSF) enhances arteriogenesis in a mouse model via a mechanism involving monocyte/macrophage mobilization. However, the arteriogenic effect of G-CSF in hypertension remains unknown. In the present study, we tested whether G-CSF affected collateral artery growth in both normotensive and hypertensive model rat. Left common carotid artery (CCA) occlusion was performed to induce hypoperfusion in the brains of Wistar rats and spontaneously hypertensive rats (SHR). G-CSF was administered subcutaneously for 5 consecutive days. The superficial angioarchitecture of the leptomeningeal anastomoses and the circle of Willis after CCA occlusion and G-CSF treatment were visualized by latex perfusion. Circulating blood monocytes and CD68-positive cells, which represented the macrophages on the dorsal surface of the brain, were counted. G-CSF enhanced leptomeningeal collateral growth in Wistar rats, but not in SHR. G-CSF increased circulating blood monocytes in both Wistar rats and SHR. The number of CD68-positive cells on the dorsal surface of the brain was increased by G-CSF in Wistar rats, but not in SHR. The increase in macrophage accumulation correlated with the observed arteriogenic effects. In conclusion, G-CSF promotes collateral artery growth in the normotensive model rat, but not in the hypertensive model rat.

1. Introduction

Reduction of the cerebrovascular reserve after major artery occlusion greatly increases the risk of ischemic stroke [1]. To maintain the cerebrovascular reserve, collateral artery growth (arteriogenesis) is essential [2]. Leptomeningeal anastomoses are the most important collateral pathway when collateral flow at the circle of Willis is not sufficient to preserve cerebral blood flow [3]. Arteriogenesis promotion could be useful as a treatment for patients with brain hypoperfusion due to major artery occlusion.

G-CSF stimulates the growth and differentiation of hematopoietic cells, and it is clinically used to treat chemotherapy-induced neutropenia. Recently, G-CSF has been reported to have arteriogenic activity [4–6]. The arteriogenic effect of G-CSF was originally reported in a mouse model of ischemic heart disease, and the underlying mechanism involved the upregulation of monocytes/macrophages [4]. We provided the first evidence that G-CSF enhances arteriogenesis in a mouse model of chronic brain hypoperfusion. In this study, G-CSF treatment provided

* Corresponding author. Tel.: +81 6 6879 3576; fax: +81 6 6878 6574.
E-mail addresses: yagita@neurol.med.osaka-u.ac.jp, yagita@medone.med.osaka-u.ac.jp (Y. Yagita).

0304-3940/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2014.01.053

powerful neuroprotection against stroke [5]. Therapeutic clinical applications of G-CSF are expected in both the cardiovascular and cerebrovascular fields, and the results of a clinical trial demonstrated that G-CSF induced arteriogenesis in coronary artery disease [7]. Clinical studies are also expected in the cerebrovascular field. However, most patients with cerebrovascular disease also develop hypertension [8,9], and the arteriogenic effects of G-CSF on a hypertensive model have not yet been determined.

Wistar rats and SHR are frequently used to compare normotensive and hypertensive states [10]. Based on the potential of G-CSF to induce the proliferation and differentiation of hematopoietic cells into white blood cells, we hypothesized that G-CSF might exert arteriogenic effects in both of these rat models. In the current study, we focused on the arteriogenic effect of G-CSF and tested its effect on monocyte/macrophage mobilization, a process that is essential to arteriogenesis. We found that G-CSF induced arteriogenesis and increased monocyte/macrophage accumulation in Wistar rats, but not in SHR.

2. Material and methods

2.1. Animals

Studies were conducted in 7–10-week-old male Wistar rats (body weight, 250–350 g) and 10–14-week-old male SHR (body weight, 250–350 g), obtained from Charles River Japan, Inc. G-CSF or vehicle was administered after left common carotid artery (CCA) occlusion. Vehicle was also administered after sham CCA operation. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Osaka University Graduate School of Medicine, Japan.

3. CCA occlusion

General anesthesia was induced by 4.0% halothane and maintained at 0.5% halothane with an open facemask. The left CCA was exposed and ligated with a silk suture. The sham CCA operation was performed in the same way without the ligation.

4. Administration of G-CSF

Recombinant human G-CSF (Kyowa-Kirin, Inc.) (100 μg/kg diluted in 0.1 mL of saline) or vehicle (0.1 mL of saline) was administered subcutaneously for 5 consecutive days immediately after CCA occlusion in Wistar rats or SHR.

5. Visualization of vessels by latex perfusion

After 7 days of CCA occlusion (n = 6–8, each group), latex perfusion was used to visualize the leptomeningeal anastomoses and the circle of Willis as previously reported [11]. The rats were anesthetized with 4.0% halothane, and the right atrium of the heart was incised to allow venous outflow. The left ventricle of the heart was cannulated and injected with 60 mL saline, and then 5 mL of latex compound (Product No. 563; Chicago Latex Products, Inc.) mixed with carbon black (Bokusai; Fueki, Inc.) (50 μL/mL) was injected. The brain was then removed from the skull, and photographs were taken of the dorsal and ventral brain surfaces. The distal MCA was identified by its branch angle and distinguished from the distal ACA or PCA. The vessel diameter of the leptomeningeal anastomoses was measured at the point where the distal MCA joined the distal ACA or PCA. The vessel diameter of the circle of Willis was measured at the proximal point where the left PCA merges with the posterior communicating artery.

6. Blood analysis

After 4 days of CCA occlusion (n = 4, each group), blood was collected by puncturing the right jugular vein. A hematology analyzer (Abaxis Vetscan HM2; Abaxis, Inc.) was used to count blood monocytes (cells/μL).

7. Immunohistochemistry

After 7 days of CCA occlusion (n = 2–3, each group), immunostaining was performed using an anti-CD68 antibody to assess macrophage accumulation on the dorsal surface of the brain. Sagittal, 20-μm-thick sections obtained between 1.5 and 3.0 mm lateral from the midline were incubated with an anti-CD68 antibody (1:100; Acris Antibodies, Inc.), and then incubated with an Alexa Fluor 488 anti-mouse IgG antibody (1:200; Molecular Probes). Ten sections were examined, and the total number of CD68-positive cells accumulated on the dorsal surface of the brain per 40 vessels was recorded. The stained sections were visualized with a microscope (Eclipse 80i; Nikon, Inc.).

8. Statistics

All data are presented as the mean ± S.E.M. Differences between multiple groups were compared by analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Nonparametric data were analyzed with the Kruskal–Wallis test followed by Dunn's multiple comparison test. A p value less than 0.05 was considered statistically significant.

9. Results

9.1. G-CSF promoted the growth of leptomeningeal anastamoses in Wistar rats

We first tested the development of leptomeningeal anastomoses in Wistar rats after 7 days of CCA occlusion by quantitative measurement of vessel diameter. Five days of G-CSF treatment significantly increased the vessel diameter of leptomeningeal anastomoses compared to the diameter in vehicle-treated rats (G-CSF, 35.0 ± 1.0 μm vs. vehicle, 31.4 ± 0.7 μm, $p < 0.05$; Fig. 1A and B). The vessel diameter of the circle of Willis was enlarged after CCA occlusion (sham CCA operation, 141.7 ± 10.7 μm vs. CCA occlusion, 209.4 ± 15.6 μm, $p < 0.05$); however, vessel diameter was not altered by G-CSF treatment (G-CSF, 223.7 ± 10.3 μm; Fig. 1C and D).

10. Leptomeningeal collateral growth was inhibited in SHR

Next, we examined whether G-CSF treatment stimulated leptomeningeal collateral growth in SHR after 7 days of CCA occlusion. G-CSF treatment did not affect leptomeningeal collateral growth (G-CSF, 28.4 ± 1.4 μm vs. vehicle, 29.5 ± 1.8 μm, n.s.; Fig. 2A and B). The vessel diameter of the circle of Willis was enlarged after CCA occlusion (sham CCA operation, 110.4 ± 1.6 μm vs. CCA occlusion, 151.2 ± 7.8 μm, $p < 0.05$); however, the diameter was not altered by G-CSF treatment (G-CSF, 151.2 ± 6.7 μm; Fig. 2C and D).

11. Circulating blood monocytes and macrophages on the dorsal surface of the brain

Previous studies clarified the pivotal role of monocytes/macrophages in collateral growth [12]. G-CSF treatment significantly increased circulating blood monocytes in Wistar rats and SHR (Wistar rats: G-CSF, 2045.0 ± 93.6/μL vs. vehicle, 457.5 ± 52.3/μL, $p < 0.01$; SHR: G-CSF, 2097.5 ± 263.3/μL vs.