Experimental Eye Research 143 (2016) 132–140

Contents lists available at ScienceDirect

Experimental Eye Research

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

Research article

Autocrine protective mechanisms of human granulocyte colony-stimulating factor (G-CSF) on retinal ganglion cells after optic nerve crush

Shun-Ping Huang [a], Kan-Tang Fang [b], Chung-Hsing Chang [c, d], Tzu-Lun Huang [e, f], Yao-Tseng Wen [g], Rong-Kung Tsai [f, g, *]

[a] Department of Molecular Biology and Human Genetics, Tzu Chi University, Hualien, Taiwan, ROC
[b] Department of Dermatology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, ROC
[c] Department of Dermatology, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, ROC
[d] Department of Dermatology, Kaohsiung Medical University, Kaohsiung, Taiwan, ROC
[e] Department of Ophthalmology, Far Eastern Memorial Hospital, Banciao District, New Taipei City, Taiwan, ROC
[f] Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan, ROC
[g] Institute of Eye Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, ROC

ARTICLE INFO

Article history:
Received 10 June 2015
Received in revised form
2 September 2015
Accepted in revised form 7 October 2015
Available online 28 October 2015

Keywords:
Human granulocyte colony-stimulating factor (G-CSF)
Autocrine
Retinal ganglion cells
G-CSF receptor
Optic nerve crush
Neuroprotection

ABSTRACT

This study investigated the role of autocrine mechanisms in the anti-apoptotic effects of human granulocyte colony-stimulating factor (G-CSF) on retinal ganglion cells (RGCs) after optic nerve (ON) crush. We observed that both G-CSF and G-CSF receptor (G-CSFR) are expressed in normal rat retina. Further dual immunofluorescence staining showed G-CSFR immunoreactive cells were colocalized with RGCs, Müller cells, horizontal and amacrine cells. These results confirm that G-CSF is an endogenous ligand in the retina. The semi-quantitative RT-PCR finding demonstrated the transcription levels of G-CSF and G-CSFR were up-regulated after ON crush injury. G-CSF treatment further increased and prolonged the expression level of G-CSFR in the retina. G-CSF has been shown to enhance transdifferentiation of the mobilized hematopoietic stem cells into tissue to repair central nervous system injury. We test the hypothesis that the hematopoietic stem cells recruited by G-CSF treatment can transdifferentiate into RGCs after ON crush by performing sublethal irradiation of the rats 5 days before ON crush. The flow cytometric analysis showed the number of CD34 positive cells in the peripheral blood is significantly lower in the irradiated, crushed and G-CSF-treated group than the sham control group or crush and G-CSF treated group. Nevertheless, the G-CSF treatment enhances the RGC survival after sublethal irradiation and ON crush injury. These data indicate that G-CSF seems unlikely to induce hematopoietic stem cell transdifferentiation into RGCs after ON crush injury. In conclusion, G-CSF may serve an endogenous protective signaling in the retina through direct activation of intrinsic G-CSF receptors and downstream signaling pathways to rescue RGCs after ON crush injury, exogenous G-CSF administration can enhance the anti-apoptotic effects on RGCs.

1. Introduction

Optic nerve (ON) injury triggers a process of initial damage and a secondary degeneration of the ON, resulting in apoptosis of retinal ganglion cells (RGCs). There is no established treatment for ON injury. Our previous works demonstrated that recombinant human granulocyte colony-stimulating factor (G-CSF) has neuroprotective effects in a rat model of ON crush, as demonstrated both structurally by RGC density and functionally by flash visual-evoked potentials (FVEPs). (Tsai et al., 2008) G-CSF may act by an anti-apoptotic mechanism involving phosphorylated-Akt (p-Akt) signaling activation as well as by inducing anti-inflammatory effects at the ON injury site. (Tsai et al., 2010).

G-CSF, a member of the cytokine family of growth factors, is

* Corresponding author. Institute of Eye Research, Buddhist Tzu Chi General Hospital, Tzu Chi University, 707 Sec. 3 Chung-Yung Rd, Hualien 97002, Taiwan, ROC.
E-mail address: rktasi@tzuchi.com.tw (R.-K. Tsai).

http://dx.doi.org/10.1016/j.exer.2015.10.010
0014-4835/

used to treat neutropenia. (Frampton et al., 1994) Administration of G-CSF results in the mobilization of hematopoietic stem cells (HSCs), primarily CD34 + HSCs, from bone marrow into the peripheral blood (PB). G-CSF has been used extensively for bone marrow reconstitution and stem cell mobilization. (Grigg et al., 1995; Weaver et al., 1993) Recently, G-CSF has been used for regeneration of non-hematopoietic tissues including skeletal muscle (Naito et al., 2009; Stratos et al., 2007) and heart. (Li et al., 2006; Okada et al., 2008; Takano et al., 2007) G-CSF facilitates functional recovery in rats after stroke. (Komine-Kobayashi et al., 2006; Schabitz et al., 2003; Schneider et al., 2005a, 2006; Sehara et al., 2007; Shyu et al., 2004) The neuroprotective effects of G-CSF occur through multiple activities, including anti-inflammation, anti-apoptosis and neurogenesis. (Schneider et al., 2005a; Shyu et al., 2004; Tsai et al., 2008) G-CSF-dependent anti-inflammatory effects occur via inhibition of inducible nitric oxide synthase (iNOS), suppression of TNF-α and reduction of interleukin-1 beta expression. (Gibson et al., 2005; Gorgen et al., 1992; Hartung et al., 1998) The binding of G-CSF to the G-CSF receptor (G-CSFR) activates a variety of intracellular signaling pathways. These include the Janus protein tyrosine kinase/signal transducer and activator of transcription (JAK/STAT), (Avalos et al., 1997; Schabitz et al., 2003) extracellular-regulated kinase (ERK) (Huang et al., 2007; Schneider et al., 2005a) and phosphatidylinositol 3-kinase/Akt (PI3K/Akt). (Dong and Larner, 2000; Naito et al., 2009; Schneider et al., 2005a, 2006) Activation of PI3k/Akt has the most powerful anti-apoptotic effect after administration of G-CSF in a stroke model. (Schneider et al., 2005a) Our previous report demonstrated that administration of G-CSF after the ON crush in rats activated the phosphorylation of AKT, but not STAT3 and ERK in the retinas. (Tsai et al., 2010) Recent studies have shown that both G-CSF and its receptor are widely expressed in the adult central nervous system (CNS) of rodents and humans. Expression is induced upon cerebral ischemia. (Hasselblatt et al., 2007; Kirsch et al., 2008; Schneider et al., 2005a) Moreover, elevated G-CSF levels were found in patients with amyotrophic lateral sclerosis, (Tanaka et al., 2006) suggesting a possible endogenous neuroprotective effect of G-CSF for neurodegenerative diseases. Similarly, G-CSFR is universally expressed in retinal cells in normal rodent retina. (Frank et al., 2009; Oishi et al., 2008) Taken together, these findings led us to hypothesize that the anti-apoptotic effects of G-CSF on RGCs after ON crush injury are an autocrine protective mechanism that activates survival signaling. Moreover, several reports have suggested that G-CSF stimulates neurogenesis (Schmidt et al., 2015; Schneider et al., 2005b) and may recruit HSCs migrating to the injured CNS. (Schneider et al., 2005a; Shyu et al., 2004; Tsai et al., 2007) One report has suggested that HSCs can differentiate into cells that express markers specific for astrocytes, macrophages/microglia, Müller cells or retinal pigment epithelial cells. (Chan-Ling et al., 2006) However, there is no direct evidence in the literature to demonstrate that HSCs mobilized by G-CSF administration can transdifferentiate into specific neurons. It is unknown whether HSCs recruited by G-CSF administration can transdifferentiate into RGCs after ON injury.

The purpose of the present study was to test the hypothesis that the neuroprotective and anti-apoptotic effects of G-CSF on RGCs after ON crush insult are involving autocrine protective mechanism. In addition, we examine whether G-CSF-mobilized HSCs have a role of neurogenesis in the injured retina.

2. Materials and methods

2.1. Animals

Seventy-two adult male Wistar rats weighing 150–180 g (7–8 weeks old) were used in this study. Rats were obtained from the breeding colony at BioLASCO Co., Taiwan. Animal care and experimental procedures were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research. The Institutional Animal Care and Use Committee (IACUC) at Tzu Chi Medical Center approved all animal experiments. All manipulations were performed with animals under general anesthesia induced by an intramuscular injection of a mixture of ketamine (40 mg/kg body weight (BW)) and xylazine (4 mg/kg BW) obtained from laboratory animal center of Tzu Chi University. In addition, topical 0.5% Alcaine eye drops (Alcon, Puurs, Belgium) were used. The rats had free access to food and water. They were maintained in cages in an environmentally controlled room that was held at a temperature of 23 ± 1 °C, a humidity of 55 ± 5% and had a 12-h light–dark cycle (light period: 7 AM–7 PM).

2.2. Sublethal irradiation and optic nerve crush injury experiment

For the experiment of sublethal irradiation, 12 adult Wistar rats were irradiated using a 4 MV waveguide Linear accelerators (Clinac 2100c, Varian, Palo Alto, CA, USA) with total doses of 6 Gy 5 days before crush experiments. After irradiation, rats were returned to their original cages with access to food and water *ad libitum*. An ON crush injury was induced as described in our previous report. (Tsai et al., 2008) Briefly, after general anesthesia and topical Alcaine eye drop application, the ON was exposed and isolated. Care was taken to avoid damaging the small vessels around the ON. A vascular clip (60 g micro-vascular clip, World Precision Instruments, FL, USA) was then applied to the ON 2 mm posterior to the globe for 30 s. After the surgery, Tobradex eye ointment (Alcon, Puurs, Belgium) was administered. The rats were kept on electric heating pads at 37 °C for recovery. The left eyes received a sham operation that entailed optic nerve exposure without the crush.

2.3. G-CSF administration

Immediately after ON crush surgery, the rats began receiving daily subcutaneous injections of recombinant human G-CSF (100 ug/kg/day in 0.2 ml of saline, 12 rats) (Takasaki Pharmaceutical Plant, Tokyo, Japan) or PBS (0.2 ml, 12 rats) as control for 5 days. We also performed intravitreal injection of G-CSF (2 ug G-CSF injection in a volume of 5 ul, 12 rats) or PBS (5 ul, 12 rats) immediately after ON crush surgery. (Tsai et al., 2008) For the experiment of RT-PCR, only one shot of G-CSF or PBS was administered.

3. Retrograde labeling of RGCs with Fluoro-Gold (FG) and densities of RGCs

The procedure is followed our previous reports (Chang et al., 2014; Tsai et al., 2010). Briefly, one week before sacrificing, the rats were anesthetized and then placed in a stereotactic apparatus (Stoelting, Wood Dale, IL, USA). An amount of 1.5 μl of 5% of FG (Fluorochrome, Denver, CO, USA) was injected into the superior colliculus on each side. One week after labeling, the eyeballs were harvested after euthanasia of the animals. The eyeballs were placed in 10% formalin and the whole retina was then carefully dissected, flattened. The retina was examined with a 400× epi-fluorescence microscope (Axioskop; Carl Zeiss Meditec. Inc., Jena, Germany) equipped with a filter set (excitation filter = 350–400 nm; barrier filter = 515 nm), as well as a digital camera (Axiocam MRm) and software (Axiovision 4.0). The retinas were examined for RGCs at a distance of 1 or 3 mm from the optic nerve head in order to provide the central and mid-peripheral RGC densities respectively. We counted eight areas randomly of 38,250 μm^2 (225 × 170 μm) each in the central (about 40% of the central area) and eight areas