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INVITED REVIEW

Efficacy of granulocyte-colony stimulating factor treatment in a rat model of anterior ischemic optic neuropathy

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Abstract

Non-arteritic anterior ischemic optic neuropathy (NA-AION) is the most common cause of acute ischemic damage to the optic nerve (ON), and the leading cause of seriously impaired vision in people over 55 years of age. It demonstrated that subcutaneous administration of Granulocyte colony-stimulating factor (G-CSF) reduces RGC death in an ON crush model in rats, and that the neuroprotective effects may involve both anti-apoptotic and anti-inflammatory processes. Our recent work shows that the protective actions of G-CSF in rAION models may involve both anti-apoptotic and anti-inflammatory processes. However, the exact rescuing mechanisms involved in the administration of G-CSF in rAION models need further investigation. In addition, further studies on the administration of G-CSF at different time intervals after the induction of rAION may be able to illustrate whether treatment given at a later time is still neuroprotective. Further, it is unknown whether treatment using G-CSF combined with other drugs will result in a synergistic effect in a rAION model. Inflammation induced by ischemia plays an essential role on the ON head in NA-AION, which can result in disc edema and compartment changes. Therefore, it is reasonable that adding an anti-inflammatory drug may enhance the therapeutic effects of G-CSF. An ongoing goal is to evaluate the novel sites of action of both G-CSF and other anti-inflammatory drugs, and to identify the functionally protective pathways to enhance RGC survival. These investigations may open up new therapeutic avenues for the treatment of ischemic optic neuropathy.

Key Words: *optic nerve; anterior ischemic optic neuropathy; retinal damage; granulocyte colony-stimulating factor; inflammatory response*

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Non-arteritic anterior ischemic optic neuropathy (NA-AION) is the most common cause of acute ischemic damage to the optic nerve (ON), and the leading cause of seriously impaired vision in people over 55 years of age. The incidence of NA-AION has been reported to range from 2.3 to 10.3 people per 100,000 individuals per year in the US (Johnson and Arnold, 1994; Hattenhauer et al., 1997). Risk factors include nocturnal hypotension, impaired autoregulation of the microvascular supply, vasculopathic occlusion, crowded disc and venous insufficiency (Hayreh, 1996; Arnold, 2003; Hayreh and Zimmerman, 2008a; Hayreh, 2009; Kerr et al., 2009). NA-AION presents clinically as a painless loss of vision in the affected eye, ON swelling (disc edema), and disruption of the normal nerve architecture, resulting in retinal ganglion cell (RGC) death through apoptosis, and permanent vision loss (Levin and Louhab, 1996). There is disagreement as to the pathogenesis and optimal management, and currently there is no effective treatment for NA-AION. In particular, the role of systemic corticosteroid therapy for NA-AION is controversial (Hayreh and Zimmerman, 2008b; Rebolleda et al., 2013). To date, no class I studies have shown any benefits from either medical or surgical treatments (Atkins et al., 2010). In addition, the characteristically high spontaneous improvement rate of NA-AION (41%; Hayreh and Zimmerman, 2008b) can easily lead to bias in clinical studies and also lead to difficulties in designing a prospective study. Other reported treatments for NA-AION in the past decade include levodopa, 0.2% brimonidine tartrate, intravitreal injection (IVI) of triamcinolone (TA), IVI of bevacizumab, and IVI of erythropoietin (Johnson et al., 2000; Faz-

zone et al., 2003; Wilhelm et al., 2006; Bennett et al., 2007; Kaderli et al., 2007; Modarres et al., 2011). However, these experimental reports were not class I clinical trials and their results were not conclusive. Therefore, larger confirmatory preclinical studies are required before an expensive randomized controlled trial can be attempted.

While the search for effective treatment strategies for NA-AION is ongoing, for pathogenic studies of NAION, a non-fatal condition, few clinical specimens have been made available (Knox et al., 2000; Tesser et al., 2003). Therefore, establishing reliable animal models of NA-AION is an alternative research strategy for preclinical trials. Recent reports using laser-induced photoactivation of intravenously administered Rose Bengal have demonstrated that observable histologic, electrophysiologic and molecular changes of the ON in a rodent model of anterior ischemic optic neuropathy (rAION) and primate NAION (pNAION) are similar to those in human NA-AION (Bernstein et al., 2011; Salgado et al., 2011).

Granulocyte colony-stimulating factor (G-CSF) is a 20-kDa glycoprotein commonly used to treat neutropenia (Frampton et al., 1994), and is known to mobilize hematopoietic stem cells (HSCs) from bone marrow into peripheral circulation. G-CSF also exhibits significant neuroprotective effects in cerebral damage models. It has been shown to facilitate a functional recovery effect in rats after stroke (Schabitz et al., 2003; Shyu et al., 2004), and to exhibit an anti-apoptotic effect through activating a variety of intracellular signaling pathways, including Janus protein tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) (Schabitz et al., 2003; Harada et al., 2005), extracellular-regulated kinase (ERK) (Schneider et al., 2005; Huang et al., 2007) and phosphatidylinositol 3-kinase/Akt (PI3K/Akt) (Dong and Larner, 2000; Komine-Kobayashi et al., 2006). In our previous studies, we demonstrated that subcutaneous administration of G-CSF reduces RGC death in an ON crush model in rats, and that the neuroprotective effects may involve both anti-apoptotic and anti-inflammatory processes (Tsai et al., 2008; Tsai et al., 2010). The neuroprotective effects of G-CSF have also been reported in models of ON axotomy (Frank et al., 2009), light induced retinal damage (Oishi et al., 2008), retinal ischemia and reperfusion (Bu et al., 2010; Shima et al., 2012) and oxygen-induced retinopathy (Kojima et al., 2011).

To further investigate the role of G-CSF in molecular changes and treatment strategies for rAION, we previously conducted a rat model of rAION using modified laser-induced photoactivation of the ON disc after intravenous administration of Rose Bengal (Chang et al., 2014). Our morphologic results showed that the RGC survival rate increased in the G-CSF-treated group compared to the PBS-treated group after rAION induction. We further demonstrated that immediate G-CSF administration after rAION induction is neuroprotective in rats (**Figure 1**). In addition, visual function assessment using flash visual evoked potentials (FVEP) also demonstrated that visual function was better preserved in G-CSF-treated rats compared to PBS-treated rats, confirming the beneficial effects of G-CSF on the ocular structures (Chang et al., 2014). The mode of RGC death in the rAION model was shown to exhibit the classical features of apoptosis, beginning at 7 days with the majority of RGC loss occurring at 21 days post-induction (Slater et al., 2008; Bernstein et al., 2011). rAION specifically targets RGCs in the retina, and does not directly damage other retinal cell types (Bernstein et al., 2003). Therefore, it is possible that a potential treatment period exists before RGC apoptosis occurs after the induction of rAION. Our TUNEL assay results showed that the application of G-CSF after rAION induction can rescue RGCs from apoptotic death and eventually preserve the survival rate of RGCs, as evidenced by our results of the density measurements of RGCs. Our previous reports also indicated that G-CSF has an anti-apoptotic effect on RGCs after ON crush injuries in rats (Tsai et al., 2008, 2010). The substantial reduction in RGC death in rAION models may thus be explained by an anti-apoptotic activity of G-CSF.

Another rescue mechanism proposed by Hartung (Hartung, 1998) states that G-CSF has prominent systemic anti-inflammatory properties which may contribute to its neuroprotective effect. In a report of human autopsies obtained 20 days after the onset of NA-AION, accumulation of Iba1t/ED1t cells (extrinsic macrophages/microglia) in ischemic areas of the ON was observed (Salgado et al., 2011). ED1-positive phagocytes found in ONs after rAION include monocytes/macrophages of hematogenous origin as well as microglia. Some ED1-positive cells have also been shown to concomitantly express the Ia antigen during Wallerian degeneration in the ON (Stoll et al., 1989). The presence of blood-borne ED1-positive cells in the ON after rAION indicates that the blood-brain barrier has been disrupted (Bernstein et al., 2011). At 4 weeks after rAION induction, our histopathological studies of the ON demonstrated disruption of the cellular columns, vacuolation of myelinated axons and accumulation of inflammatory cells in the PBS-treated rats, with less edema, less inflammatory cell infiltration and greatly preserved architecture of the ON in G-CSF-treated rats. Our results further showed that ED1-positive macrophage/microglia accumulation at the ON lesion site was attenuated in the G-CSF-treated and rAION-inducted rats, suggesting that the immediate administration of G-CSF may have an anti-inflammatory effect on injured ONs after rAION induction. Macrophage/microglia accumulation can activate inflammatory mediators such as tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and cytokine expressions. G-CSF has been reported to have anti-inflammatory actions mediated by the inhibition of TNF-a, by decreasing iNOS activity (Gorgen et al., 1992), and by reducing interleukin-1β expression (Gibson et al., 2005). Taken together, this indicates that G-CSF can decrease the inflammatory response at the ON by inhibition of the above inflammatory mediators, and that it may protect RGCs from secondary degeneration.

Recent studies have shown that both G-CSF and its receptors are widely expressed in the adult central nervous system and retinas (Hasselblatt et al., 2007; Oishi et al., 2008; Tsai et

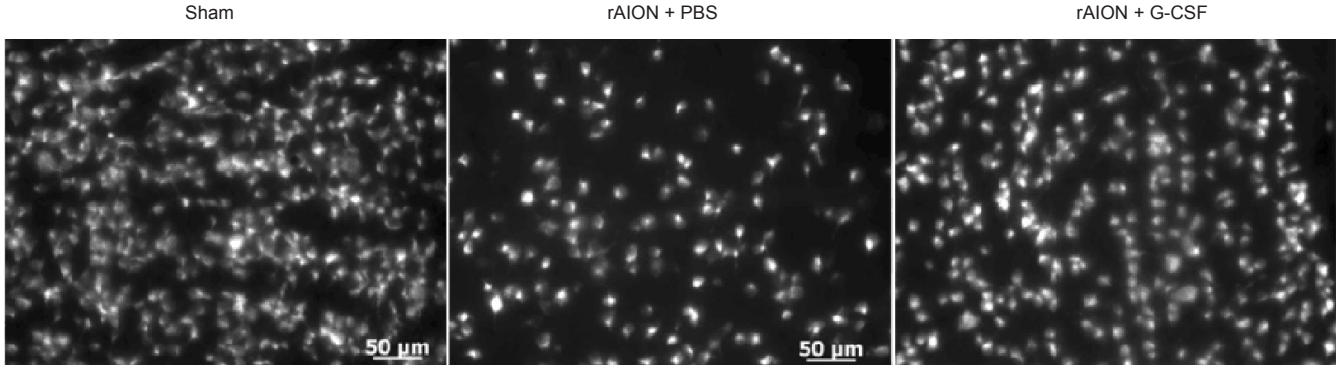

Figure 1 Flat preparations of retinas and morphometry of retinal ganglion cells (RGCs) by FluoroGold retrograde labeling at 4 weeks after rodent anterior ischemic optic neuropathy (rAION) induction.
The survival rate of the RGCs in the central retinas in the granulocyte colony-stimulating factor (G-CSF) treated rats was significantly higher than in the PBS-treated rats (71.4% *vs.* 33.2%, $P < 0.01$).

al., 2010). It has also been suggested that the autocrine protective mechanism of G-CSF is a protective mechanism for neurons (Schneider et al., 2005; Oishi et al., 2008; Tsai et al., 2010). Besides direct reaction with its receptors, G-CSF has been reported to induce the release of neurotrophic factors from glial cells (Solaroglu et al., 2006), and to have trophic effects on neuronal cells (Konishi et al., 1993). Furthermore, the application of G-CSF is known to result in the mobilization of HSCs to the peripheral blood. Trafficking of HSCs to the injured neurons mediated by stromal cell-derived factor 1a has been reported to lead to the production of trophic factors that contribute to anti-inflammation, survival of the damaged neural tissue (Majka et al., 2001; Tsai et al., 2008), and neurogenesis (Shyu et al., 2004; Schneider et al., 2005).

Our recent work shows that the protective actions of G-CSF in rAION models may involve both anti-apoptotic and anti-inflammatory processes. However, the exact rescuing mechanisms involved in the administration of G-CSF in rAION models need further investigation. In addition, further studies on the administration of G-CSF at different time intervals after the induction of rAION may be able to illustrate whether treatment given at a later time is still neuroprotective. Further, it is unknown whether treatment using G-CSF combined with other drugs will result in a synergistic effect in a rAION model. Inflammation induced by ischemia plays an essential role on the ON head in NA-AION, which can result in disc edema and compartment changes. Therefore, it is reasonable that adding an anti-inflammatory drug may enhance the therapeutic effects of G-CSF. An ongoing goal is to evaluate the novel sites of action of both G-CSF and other anti-inflammatory drugs, and to identify the functionally protective pathways to enhance RGC survival. These investigations may open up new therapeutic avenues for the treatment of ischemic optic neuropathy.

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