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

**Funding:** This work was funded by a National Science Council Grant from the Taiwanese Government NSC100-2314-B-303-005.

Huang SP, Tsai RK. Efficacy of granulocyte-colony stimulating factor treatment in a rat model of anterior ischemic optic neuropathy. Neural Regen Res. 2014;9(16):1502-1505.
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 (aAION) 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 Iba1/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 1a 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-induced 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-α, 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 retina (Hasselblatt et al., 2007; Oishi et al., 2008; Tsai et
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. Induction mediated 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.

Acknowledgments: We thanks Mr. Higgins M for his work in English editing.

Author contributions: Huang SP was the main developer of this manuscript. Tsai RK revised the manuscript. Both of these two authors approved the final version of this manuscript.

Conflicts of interest: None declared.

References
Arnold AC (2003) Pathogenesis of nonarteritic anterior ischemic optic neuropathy. J Neuroophthalmol 23:157-163.
Atkins EJ, Bruce BB, Newman NJ, Bioussse V (2010) Treatment of nonarteritic anterior ischemic optic neuropathy. Surv Ophthalmol 55:47-63.
Bennett JL, Thomas S, Olson JL, Mandava N (2007) Treatment of nonarteritic anterior ischemic optic neuropathy with intravitreal bevacizumab. J Neuroophthalmol 27:238-240.
Bernstein SL, Guo Y, Kelm SE, Flower RW, Johnson MA (2003) Functional and cellular responses in a novel rodent model of anterior ischemic optic neuropathy. Invest Ophthalmol Vis Sci 44:4153-4162.
Bernstein SL, Johnson MA, Miller NR (2011) Nonarteritic anterior ischemic optic neuropathy (NAION) and its experimental models. Prog Retin Eye Res 30:167-187.
Bu P, Basith B, Stubbs EB Jr, Perlman JI (2010) Granulocyte colony-stimulating factor facilitates recovery of retinal function following retinal ischemic injury. Exp Eye Res 91:104-106.
Chang CH, Huang TL, Huang SP, Tsai RK (2014) Neuroprotective effects of recombinant human granulocyte colony-stimulating factor (G-CSF) in a rat model of anterior ischemic optic neuropathy (rAION). Exp Eye Res 118:109-116.
Dong F, Larner AC (2000) Activation of Akt kinase by granulocyte colony-stimulating factor (G-CSF): evidence for the role of a tyrosine kinase activity distinct from the Janus kinases. Blood 95:1656-1662.
Fazzone HE, Kupersmith MJ, Leibmann I (2003) Does topical brimonidine tartrate help NAION? Br J Ophthalmol 87:1193-1194.
Frampton JE, Lee CR, Faulds D (1994) Filgrastim. A review of its pharmacological properties and therapeutic efficacy in neutropenia. Drugs 48:731-760.
Frank T, Schlachetzki J, Goricke B, Meuer K, Rohde G, Dietz GP, Bahr M, Schneider A, Weishaupt JH (2009) Both systemic and local application of granulocyte-colony stimulating factor (G-CSF) is neuroprotective after retinal ganglion cell axotomy. BMC Neurosci 10:49.
Gibson CL, Jones NC, Prior MJ, Bath PM, Murphy SP (2005) G-CSF suppresses edema formation and reduces interleukin-1beta expression after cerebral ischemia in mice. J Neuropathol Exp Neurol 64:763-769.
Görgen I, Hartung T, Leist M, Niehorster M, Tieg S, Uhlig S, Weitzen F, Wendel A (1992) Granulocyte colony-stimulating factor treatment protects rodents against lipopolysaccharide-induced toxicity via suppression of systemic tumor necrosis factor-alpha. J Immunol 149:918-924.
Harada M, Qin Y, Takano H, Minamino T, Zou Y, Toko H, Ohtsuka M, Matsura K, Sano M, Nishi J, Iwanga K, Akazawa H, Kinieda T, Zhu W, Hasegawa H, Kunisada K, Nagai T, Nakaya H, Yamauchi-Takahara K, Komuro I (2005) G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med 11:305-311.

Hartung T (1998) Anti-inflammatory effects of granulocyte colony-stimulating factor. Curr Opin Hematol 5:221-225.

Hasselblatt M, Jeibmann A, Riesmeier B, Maintz D, Schabitz WR (2007) Granulocyte-colony stimulating factor (G-CSF) and G-CSF receptor expression in human ischemic stroke. Acta Neuropathol 113:45-51.

Hattenhauer MG, Leevitt JA, Hodge DO, Grill R, Gray DT (1997) Incidence of nonarteritic anterior ischemic optic neuropathy. Am J Ophthalmol 123:103-107.

Hayreh SS (1996) Acute ischemic disorders of the optic nerve: pathogenesis, clinical manifestations and management. Ophthalmol Clin North Am 9:407-442.

Hayreh SS (2009) Pathogenesis of nonarteritic anterior ischemic optic neuropathy. Arch Ophthalmol 127:1082-1083.

Hayreh SS, Zimmerman MB (2008a) Nonarteritic anterior ischemic optic neuropathy: refractive error and its relationship to cup/disc ratio. Ophthalmology 115:2275-2281.

Hayreh SS, Zimmerman MB (2008b) Non-arteritic anterior ischemic optic neuropathy: role of systemic corticosteroid therapy. Graefes Arch Clin Exp Ophthalmol 246, 1029-1046.

Huang HY, Lin SZ, Kuo JS, Chen WF, Wang MJ (2007) G-CSF protects dopaminergic neurons from 6-OHDA-induced toxicity via the ERK pathway. Neurobiol Aging 28:1258-1269.

Johnson LN, Arnold AC (1994) Incidence of nonarteritic and arteritic anterior ischemic optic neuropathy. Population-based study in the state of Missouri and Los Angeles County, California. J Neuroophthalmol 14:38-44.

Johnson LN, Garey ME, Krohel GB, Madsen RW (2000) Levodopa may improve vision loss in recent-onset, nonarteritic anterior ischemic optic neuropathy. Ophthalmology 107:521-526.

Kaderli B, Avci R, Yucel A, Guler K, Gelskin O (2007) Intravitreal triamcinolone improves recovery of visual acuity in nonarteritic anterior ischemic optic neuropathy. J Neuroophthalmol 27:164-168.

Kerr NM, Chew SS, Danesh-Meyer HV (2009) Non-arteritic anterior ischaemic optic neuropathy: a review and update. J Clin Neurosci 16:994-1000.

Knox DL, Kerrison JB, Green WR (2000) Histopathologic studies of ischemic optic neuropathy. Trans Am Ophthalmol Soc 98:203-220.

Koijima H, Otani A, Oishi A, Makijima Y, Nakagawa S, Yoshimura N (2011) Granulocyte colony-stimulating factor attenuates oxidative stress-induced apoptosis in vascular endothelial cells and exhibits functional and morphologic protective effect in oxygen-induced retinopathy. Blood 117:1091-1100.

Komine-Kobayashi M, Zhang N, Liu M, Tanaka R, Hara H, Osaka A, Mochizuki H, Mizuno Y, Urabe T (2006) Neuroprotective effect of recombinant human granulocyte colony-stimulating factor in transtemporal focal ischemia of mice. J Cereb Blood Flow Metab 26:402-413.

Konishi Y, Chui DH, Hirose H, Kunishita T, Tabira T (1993) Trophic effect of erythropoietin and other hematopoietic factors on central cholinergic neurons in vitro and in vivo. Brain Res 609:29-35.

Levin LA, Louhab A (1996) Apoptosis of retinal ganglion cells in anterior ischemic optic neuropathy. Arch Ophthalmol 114:488-491.

Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z, Kowalska MA, Gewirtz AM, Emerson SG, Ratajczak MZ (2001) Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. Blood 97:3075-3085.

Modarres M, Falavarjani KG, Nazari H, Sanjari MS, Aghahomamadi F, Homaii M, Samiy N (2011) Intravitreal erythropoietin injection for the treatment of non-arteritic anterior ischaemic optic neuropathy. Br J Ophthalmol 95:992-995.

Oishi A, Otani A, Sasahara M, Kojima H, Nakamura H, Yodoi Y, Yoshimura N (2008) Granulocyte colony-stimulating factor protects retinal photoreceptor cells against light-induced damage. Invest Ophthalmol Vis Sci 49:5629-5635.

Rebolleda G, Perez-Lopez M, Casas LP, Contreras I, Munoz-Negrete FJ (2013) Visual and anatomical outcomes of non-arteritic anterior ischemic optic neuropathy with high-dose systemic corticosteroids. Graefes Arch Clin Exp Ophthalmol 251:255-260.

Salgado C, Vilsen F, Miller NB, Bernstein SL (2011) Cellular inflammation in nonarteritic anterior ischemic optic neuropathy and its primate model. Arch Ophthalmol 129:1583-1591.

Schabitz WR, Kollmar R, Schwaninger M, Juettler E, Bardutzky J, Scholze MN, Sommer C, Schwab S (2003) Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. Stroke 34:745-751.

Schneider A, Kruger C, Steigleder T, Weber D, Pitzer C, Laage R, Aronowski J, Maurer MH, Gaslier N, Mier W, Hasselblatt M, Kollmar R, Schwab S, Sommer C, Bach A, Kuhn HG, Schabitz WR (2005) The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. J Clin Invest 115:2083-2098.

Shima C, Adachi Y, Minamino K, Okigaki M, Shi M, Imai Y, Yano S, Takahashi K, Ikehara S (2012) Neuroprotective effects of granulocyte colony-stimulating factor on ischemia-reperfusion injury of the retina. Ophthalmic Res 48:199-207.

Shyu WC, Lin SZ, Yang HI, Tseng YS, Pang CY, Yen PS, Li H (2004) Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells. Circulation 110:1847-1854.

Slater B, Mehrabian Z, Guo Y, Hunter A, Bernstein SL (2008) Rodent anterior ischemic optic neuropathy (rAION) induces regional ganglion cell apoptosis with a unique temporal pattern. Invest Ophthalmol Vis Sci 49:3671-3676.

Solaroglu I, Tsubokawa T, Cahill J, Zhang JH (2006) Anti-apoptotic effect of granulocyte-colony stimulating factor after focal cerebral ischemia in the rat. Neuroscience 143:963-974.

Stoll G, Trapp BD, Griffin JW (1989) Macrophage function during Wallerian degeneration of rat optic nerve: clearance of degenerating myelin and la expression. J Neurosci 9:2327-2335.

Tesser RA, Niendorf ER, Levin LA (2003) The morphology of an infarct in nonarteritic anterior ischemic optic neuropathy. Ophthalmology 110:2031-2035.

Tsai RK, Chang CH, Wang HZ (2008) Neuroprotective effects of recombinant human granulocyte colony-stimulating factor (G-CSF) in neurodegeneration after optic nerve crush in rats. Exp Eye Res 87:242-250.

Tsai RK, Chang CH, Shiu MM, Huang ZL (2010) Anti-apoptotic effects of human granulocyte colony-stimulating factor (G-CSF) on retinal ganglion cells after optic nerve crush are PI3K/AKT-dependent. Exp Eye Res 90:537-545.

Wilhelm B, Ludtke H, Wilhelm H (2006) Efficacy and tolerability of 0.2% brimonidine tartrate for the treatment of acute non-arteritic anterior ischemic optic neuropathy (NAION): a 3-month, double-masked, randomised, placebo-controlled trial. Graefes Arch Clin Exp Ophthalmol 244:551-558.