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Research report

Granulocyte-colony stimulating factor protects against endoplasmic reticulum stress in an experimental model of stroke

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ABSTRACT

Granulocyte-colony stimulating factor (G-CSF) is an endogenous growth factor that exhibits a diverse range of neuroprotective mechanisms against a variety of neurological disorders including ischemic stroke. We investigated the anti-apoptotic mechanisms of G-CSF against endoplasmic reticulum (ER) stress induced apoptosis. Sprague-Dawley rats were subjected to transient occlusion of the middle cerebral artery (MCAO) for 90 min. Rats were injected with G-CSF (n = 15; 50 μg/kg body weight s.c.) for 4 days, starting 24 h post-MCAO and brains were harvested after 4 days reperfusion (n = 16). Key proteins in ER stress apoptosis were analyzed by immunoblotting. G-CSF reduced infarct volume to 53% and improved neurological deficits. G-CSF treatment significantly ($P < .05$) attenuated the expression of proteins involved in ER stress apoptosis pathway; ATF4, ATF6, p-p38MAPK, pJNK and CHOP. G-CSF treatment also re-established ER homeostasis evident by the reduction of the intraluminal ER stress sensor, GRP78 as well as reducing the overall cellular stress level protein, HSP27. G-CSF also up-regulated anti-apoptotic proteins pAKT and Bcl-2 while down-regulated the pro-apoptotic protein Bax. G-CSF exerts neuroprotection from cerebral ischemia through the preservation of the ER, resulting in the attenuation of pro-apoptotic proteins and the potentiation of anti-apoptotic proteins.

1. Introduction

Stroke is one of the world's leading causes of death and disability. One common type of stroke is ischemic stroke, and 85% of cerebral stroke occurs as this type of stroke (Shiber et al., 2010). An insufficient supply of oxygen and glucose result in an ischemic cascade including endoplasmic reticulum (ER) dysfunction (Durukan and Tatlisumak, 2007). Proteins are synthesized, folded and undergo post-translational modification in the ER. Optimum protein folding in the ER is provided by calcium (Ca^{2+}), ER chaperone proteins and an oxidizing environment within the lumen of the ER. A perturbation of all or any one of these factors leads to the accumulation of unfolded proteins; a condition termed ER stress (Sovolyova et al., 2014). In order to overcome ER stress, an ER stress mechanism, referred to as the "unfolded protein response" (UPR) is initiated (Cao and Kaufman, 2012). Ischemia triggers the accumulation of unfolded proteins in the ER, with the subsequent activation of the unfolded protein response (UPR). The UPR involves the detection of unfolded protein by the intraluminal ER chaperone; glucose regulated protein 78 (GRP 78), which then dissociates from the three ER stress transmembrane sensors; double-stranded RNA-activated protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring kinase 1 (IRE1), leading to their activation. Downstream targets of the PERK, ATF6 and IRE1 pathways will upregulate UPR genes and ER-associated degradation (ERAD) genes that will degrade the unfolded protein in the ER (Pan et al., 2012). However in prolonged ER stress, the three pathways upregulate the expression of the pro-apoptotic transcription factor, C/EBP homologous protein (CHOP) (Ron and Walter, 2007) which favors apoptosis by downregulating the anti-apoptotic protein, Bcl-2 while upregulating pro-apoptotic

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proteins; Bim, and Bax (Mohammad-Gharibani et al., 2014; Ghosh et al., 2012; McCullough et al., 2001; Plácido et al., 2014; Puthalakath et al., 2007). The ischemic brain injury includes the core, an area that experiences a severe reduction in blood flow and therefore exists in a continuum between apoptosis and necrosis cell death (Martin, 2010) and may still be affected by therapy (Modi et al., 2014; Mohammad-Gharibani et al., 2014; Sun et al., 2011). The penumbra (the area surrounding the core) is metabolically active due to collateral blood flow and is very susceptible to therapeutic interventions (Agarwal et al., 2013). With such small progress towards the development of stroke treatments, it is therefore important to develop efficacious neuroprotective agents to treat stroke.

Granulocyte-colony stimulating factor (G-CSF) is a 19.6 kDa glycoprotein that belongs to the family of cytokine hematopoietic growth factors (Christopher et al., 2011). G-CSF regulates the generation, proliferation, survival, and maturation of neutrophilic granulocytes and induces their mobilization from bone marrow to the peripheral blood (Bajrami et al., 2016; Knudsen et al., 2011). It is FDA approved and presently used in clinical practice

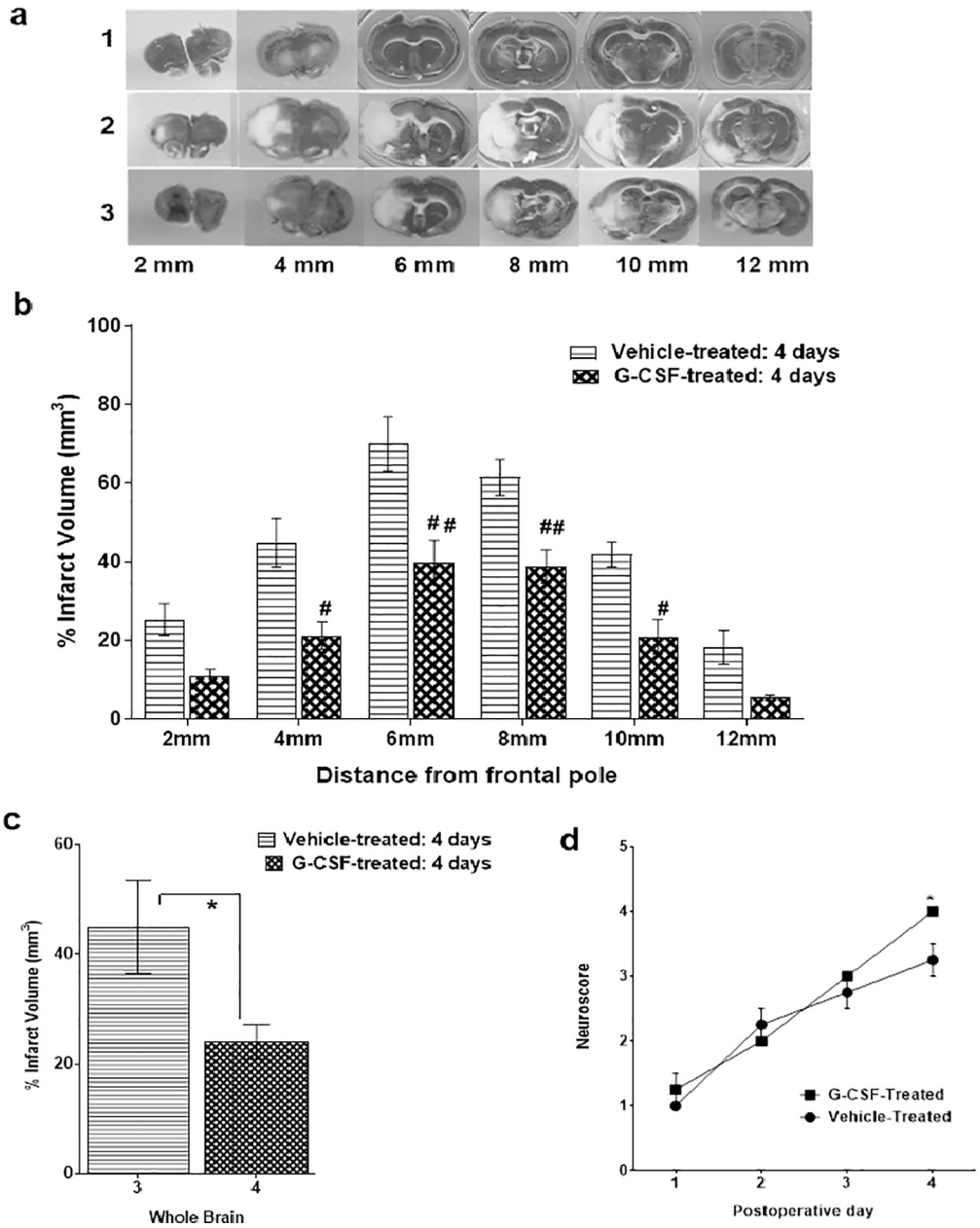

Fig. 1. Histological assessment on infarct volume and neuro-score evaluation of the effect of G-CSF in transient focal ischemia. (a) Coronal brain sections (2, 4, 6, 8, 10, and 12 mm from the frontal pole) stained with 2% TTC (white areas reflect a lack of staining and hence a loss of viable tissue). **1** – Sham operated rats (n = 9); MCAO animals that were either **2** – vehicle-treated (4 days reperfusion, n = 16) and **3** – G-CSF-treated (4 days reperfusion, n = 15). (b) Quantitative analysis of infarct volume on each coronal brain sections (2, 4, 6, 8, 10, and 12 mm) for 4 day vehicle-treated (n = 12) and 4 days GCSF-treated (n = 15) (mean ± SEM # # P < .01 and # P < .05 versus 4 days vehicle- treated for corresponding brain sections, two way ANOVA, Tukey post hoc test) (c) Quantitative analysis of infarct volume on whole brain for 4 days vehicle-treated (n = 8) and 4 days G-CSF-treated (n = 14) (mean ± SEM # P < .05 versus 4 days vehicle-treated, Student's *t*-test, two tail). (d) Neurological recovery during a 4-day examination postoperative in vehicle treated (n = 8) and G-CSF treated rats (n = 8). All scores were noted, the lowest score observed was the score assigned as the rat's behavior for that day. Sham operated rats exhibited normal behavior and were excluded from the analysis. Both G-CSF and vehicle treated rats showed improved neurological score with time. The G-CSF animal showed greater and better sustained improvement in neurological score which was significantly different than vehicle animals on day 4 (P < .05). Two way ANOVA, Sudick post hoc.