CHARLES E. SCMIDT COLLEGE OF MEDICINE

Neuroprotective Mechanism of Granulocyte-Colony Stimulating Factor against focal cerebral ischemia

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Cerebral ischemic stroke is one of the world's leading causes of death and disability. Current treatment remains mostly ineffective and only allows a small window of 4.5 hours for effective treatment. It is therefore imperative to develop novel and efficacious therapies that will expand the treatment window. Granulocyte-Colony Stimulating Factor (G-CSF) is a member of the cytokine family of growth factor, crosses the blood-brain barrier and is already FDA approved for the treatment of neutropenia. Although many studies have reported on the neuroprotective property of G-CSF in focal ischemia only a few have focused on its molecular mechanisms. The action of G-CSF is mediated through its receptor, G-CSFR, which activates the PI3-K/AKT pathway as one of its major intracellular pathways. Male Sprague-Daley rats were subjected to transient 90 minutes ischemia via the occlusion of the proximal middle cerebral artery (pMCAO) using the intraluminal technique. Twenty-four hours (24 hrs) post-reperfusion rats were given the first dose of G-CSF (50ug/kg body weight, subcutaneously) followed by a daily administration of the same dose for three days. Infarct volume of rat brains were measured by the 2,3,5-Triphenyl-2-H-tetrazolium (TTC) method while expression of anti-apoptotic molecules; phosphorylated AKT, Bcl_2 and pro-apoptotic molecules; Bax, CHOP were analyzed by western blotting. G-CSF significantly reduces infarct volume, increases the ratio of Bcl_2 to Bax, increases pAKT and reduces the expression of CHOP. This validates the neuroprotective action of G-CSF and highlights some of the key molecules in its neuroprotective mechanism.

Neuroprotective Mechanism of Granulocyte-Colony Stimulating Factor against Focal Cerebral Ischemia.

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Abstract

Cerebral ischemic stroke is one of the world's leading causes of death and disability. Current treatment remains mostly ineffective and only allows a small window of 4.5 hours for effective treatment. It is therefore imperative to develop novel and efficacious therapies that will expand the treatment window. Granulocyte-Colony Stimulating Factor (G-CSF) is a member of the cytokine family of growth factor, crosses the blood-brain barrier and is already FDA approved for the treatment of neutropenia. Although many studies have reported on the neuroprotective property of G-CSF in focal ischemia only a few have focused on its molecular mechanisms. The action of G-CSF is mediated through its receptor, G-CSFR, which activates the phosphatidylinositol 3-kinases/AKT (PI3-K/AKT) pathway as one of its major intracellular pathways. Male Sprague-Daley rats were subjected to transient 90 minutes ischemia via the occlusion of the proximal middle cerebral artery (pMCAO) using the intraluminal technique. Twenty-four hours (24 hrs) post-reperfusion rats were given the first dose of G-CSF (50ug/kg body weight, subcutaneously) followed by a daily administration of the same dose for three days. Infarct volume of rat brains were measured by the 2,3,5-Triphenyl-2-H-tetrazolium (TTC) method while expression of anti-apoptotic molecules; phosphorylated AKT (pAKT), B cell leukemia/lymphoma 2 (Bcl-2) and pro-apoptotic molecules; Bcl-2 associated X protein (Bax), C/EBP-homologous protein (CHOP), were analyzed by western blotting. G-CSF significantly reduces infarct volume, increases the ratio of Bcl-2 to Bax, increases pAKT and reduces the expression of CHOP. This validates the neuroprotective action of G-CSF and highlights some of the key molecules in its neuroprotective mechanism.

Materials and Method

Male Sprague-Daley rats (250 -300 g) were subjected to transient 90 minutes ischemia via the occlusion of the proximal middle cerebral artery (pMCAO) using the intraluminal technique. Twenty-four hours (24 hrs) post-reperfusion rats were given the first dose of G-CSF (50ug/kg body weight, subcutaneously) followed by a daily administration of the same dose for three days. Infarct volume of rat brains were measured by the 2,3,5-Triphenyl-2-H-tetrazolium (TTC) method while expression of anti-apoptotic molecules; B cell leukemia/lymphoma 2 (Bcl-2) and pro-apoptotic molecules; Bcl-2 associated X protein (Bax), C/EBP-homologous protein (CHOP), were analyzed by western blotting.

Results

Fig 1. Induction of Focal Cerebra Ischemia

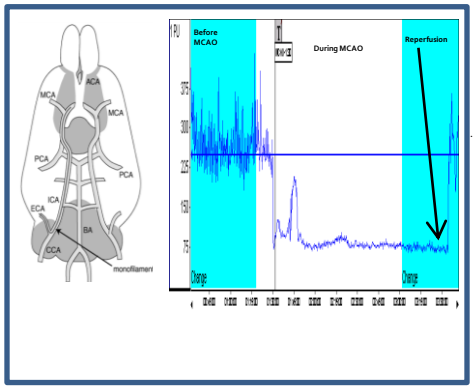
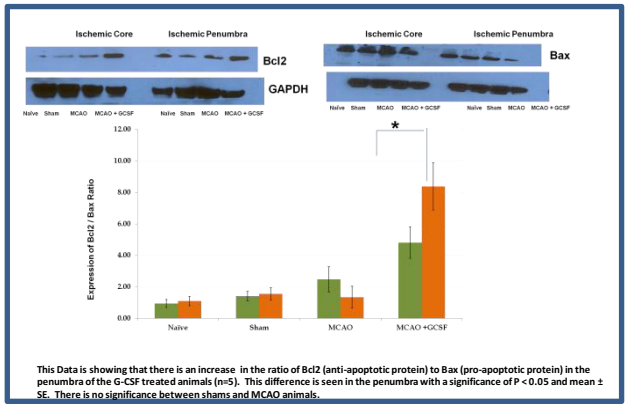


Fig 3. G-CSF effect's on Bcl2/Bax Ratio



This Data is showing that there is an increase in the ratio of Bcl2 (anti-apoptotic protein) to Bax (pro-apoptotic protein) in the penumbra of the G-CSF treated animals (n=5). This difference is seen in the penumbra with a significance of $P < 0.05$ and mean ± SE. There is no significance between shams and MCAO animals.

Fig 2. Analysis of Infarct volume with TTC

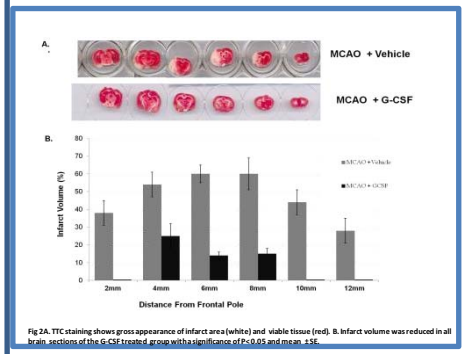
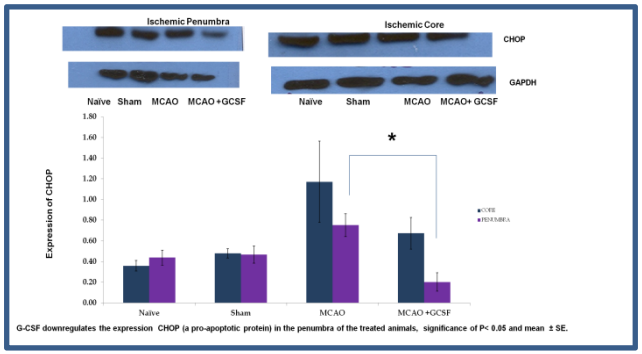


Fig 2A. TTC staining shows gross appearance of infarct area (white) and viable tissue (red). B. Infarct volume was reduced in all brain sections of the G-CSF treated group with a significance of $P<0.05$ and mean ±SE.

Fig4. G-CSF effect's on CHOP



G-CSF downregulates the expression CHOP (a pro-apoptotic protein) in the penumbra of the treated animals, significance of $P< 0.05$ and mean ± SE.

Conclusion

We can conclude that G-CSF is able to reduce ischemic volume and upregulates key anti-apoptotic proteins, such as Bcl2 while downregulating anti-apoptotic one, CHOP. This a very good indication that G-CSF neuroprotective mechanism is through ER –stress pathway since CHOP is a ER –stress marker. Since G-CSF upregulates Bcl2, further research need to be done to observes if this correlates with reduced release of cytochrome C from the mitochondria which would implies that G-CSF not only reduces ER-stress apoptosis but also apoptosis due to mitochondrial dysfunction.

Acknowledgements

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