Title: Granulocyte Colony-Stimulating Factor (GCSF) protects vulnerable neonatal brain regions from hypoxic injury following brief, repeated apnea

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Introduction: Granulocyte colony-stimulating factor (G-CSF), a cytokine growth factor, blocks inflammation, excitotoxicity and apoptosis. Our purpose was to investigate the effect of G-CSF on the expression of key proteins which are known to signal the initiation (Bax, Caspase-3) or inhibition (CREB, p-Akt, and Bcl-2) of apoptosis in the neonatal brain when subjected to severe, repeated, intermittent apnea.

Methods: Halothane and fentanyl anesthetized, pancuronium paralyzed, mechanically ventilated newborn piglets were randomized to receive 17ug/kg intravenous G-CSF or placebo 1 hour prior to apneic events. The piglets then underwent 10 repeated (3.5 minutes) episodes of apnea, with 21% oxygen resuscitation for 15 minutes between episodes. Following 6 hours of recovery, the piglets were euthanized and the brain regions dissected and frozen for analysis. Expression of Bax, Caspase-3 or neuronal protection proteins (Bcl-2, p-Akt, p-CREB) were measured by Western Blot. Results are mean ±SD % compared to sham operated control animals. Analysis was by Student's t-test.

Results: In the *striatum*, G-CSF treated animals had lower Bax and Caspase-3 expression (116±6% vs. 133±11%, $p<0.05$ and 180±36% vs. 262±33%, $p<0.01$) and higher Bcl-2 (198±22% vs.122±4%, $p<0.05$), p-Akt (248±35% vs. 175±14%, $p<0.01$), and p-CREB (184±36% vs. 158±16%, $p<0.01$) expression. The Bcl-2/Bax ratio was increased 2 fold (1.71 vs. 0.88, $p<0.05$).
In the *hippocampus*, Bax expression was lower (87±7% vs. 100±11%, $p<0.05$) and p-Akt higher (170±35% vs. 108±35%, $p<0.05$) in G-CSF treated animals.
In the frontal cortex and midbrain, regions more resistant to hypoxic injury, there were no significant differences between the G-CSF treated and the control groups.

Discussion: In this neonatal piglet model of repeated, severe, intermittent apnea induced hypoxia and 21% oxygen resuscitation, pre-injury treatment with G-CSF provided significant neuronal protection for the selectively vulnerable regions of the brain. Further investigation into the mechanism and clinical benefits of pre-apneic treatment with G-CSF seems warranted.