Regulated Expression of VEGF by Stem Cells to Improve Outcome After Ischemic Stroke in Infant Piglets. John Kuluz, T Huang, D He, M Ramirez, R De Los Santos, K Padgett, B Watson, D Dietrich and K Webster. University of Miami Miller School of Medicine, Miami, FL 33101

For the past 7 years our lab has focused on developing a new model of pediatric ischemic stroke in infant piglets, and more recently evaluating whether adipose-derived stem cells (ADSCs), genetically modified for regulated expression of vascular endothelial growth factor (VEGF), improve outcome after stroke in infant piglets. Ischemic stroke was produced in male infant piglets (2 to 4 weeks old) by photothrombotic occlusion of the middle cerebral artery as previously described. We hypothesized that modified ADSCs administered by lumbar puncture after stroke onset, migrate to the infarct, reduce cellular injury and enhance tissue and functional recovery.

Ischemic stroke in piglets: Stroke is produced by photothrombotic occlusion of the MCA, resulting in reproducible focal cerebral ischemia followed by a consistent volume of infarction which is moderate in size and involves both white and gray matter. The mass of brain tissue with ischemic levels of blood flow increased from 16% at 15 minutes to 25% at 4 hours, and to 40% of the ischemic hemisphere at 24 hours post-MCAO. Newborn piglets had greater recovery of rCBF than did infants and young adults. We extended survival of infant piglets for up to 7 days post-MCAO and measured serial MRIs, histopathology and functional outcome. The volume of infarction increased from 9.6 ± 2.4% of ischemic hemisphere at 4 hours to 35.7 ± 9.3% at 24 hours post-stroke ($p<0.05$). Between 1 and 7 days after stroke infarction did not change significantly, although the infarct tended to be smaller at 7 days (30%). Neurological score was lowest at 24 hours but gradually recovered, although not completely, at 7 days.

Preparation and administration of VEGF-modified ADSCs: ADSCs were easily harvested and expanded from a small amount of fat from infant piglets. These cells met the criteria of stem cells in that they differentiated into multiple cell types, including adipocytes, osteocytes and chondroblasts. Piglet ADSCs were transfected with a unique genetic combination of regulatory elements such that VEGF expression occurs only under conditions of severe hypoxia. Modified ADSCs were shown to secrete VEGF only when exposed to hypoxia in vitro, and maintained a stable secretion of VEGF for up to 2 weeks under hypoxic conditions. Modified ADSCs protected primary cultures of cortical neurons from ischemic injury in a transwell insert system by reducing neuronal death. VEGF-ADSCs were also found to promote neurogenesis and axon outgrowth of neural stem cells under hypoxic condition (0.5% oxygen tension) demonstrated by the mature neuronal marker, β-tubulin III. The protective effects of ADSCs may have been due to neurotrophic effects since we measured an increase in the production of neural growth factor (NGF) by ADSCs in response to injured astrocyte media.

For the in vivo studies, we injected 1×10^6 BrdU-labeled ADSCs in 1 ml PBS into piglets 24h after stroke via lumber puncture. Piglets were sacrificed 1, 3 and 10 days later (n=5 total). After LP injection, ADSCs migrated to the zone of infarction, secreted VEGF within 24h, and survived up to 10 d without evidence of rejection on histopathology. ADSCs appeared to promote angiogenesis and reduce neuronal apoptosis after stroke as demonstrated by higher blood vessel density and fewer caspase-3 positive cells within the infarct lesion. Some ADSCs in the infarction also expressed NGF. These preliminary studies show that ADSCs can be easily harvested and expanded, modified to produce VEGF in ischemic tissue, and may promote recovery after stroke through reduction in cell death and enhanced angiogenesis. Injection of ADSCs via lumbar puncture appears to be an effective method of administration which carries less risk of CNS injury compared to direct tissue implantation. Future studies will examine the effect of modified ADSCs on functional and histopathological outcome following ischemic stroke in piglets.