the cerebellum and hippocampus after 30 days post-transplant. Our results demonstrate that implanted human, brain-derived fetal stem cells reduced the ataxic symptoms and extended longevity in the sHW rat, suggesting future clinical benefit for the utilization of these stem cells in treatments of neurodegenerative diseases.

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PROTECTION OF BRAIN CELLS IN ORGANOTYPIC SLICE CULTURES EXPOSED TO OXYGEN AND GLUCOSE DEPRIVATION IS SPECIFICALLY MEDIATED BY CORD BLOOD CD14+ CELLS

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We are developing clinical products derived from human cord blood [CB] mononuclear cells [CBMC] to protect the brain from acute hypoxic injury. We have standardized an organotypic mouse brain slice culture model to identify CBMC subpopulations that protect brain cells from death following oxygen-glucose deprivation [OGD]. To prepare CBMC, <2day old CB units were centrifuged on Ficoll®, treated with NH_4Cl, and washed in medium. Brain slice [300μm] cultures established from P1 or P2 C57BL/6J mice were maintained for 8-10 days on membrane filters over serum free medium under normoxic conditions, subjected to OGD [glucose free medium in <1 % O_2; 1 hour], and then returned to normal conditions. CBMC were then added on top of the slices. Co-cultures were maintained 72h. OGD induced death was measured by propidium iodide staining. Adding CBMC reduced cell death in a dose dependent manner; 25,000 CBMC reduced cell death 80 ± 5% [mean ± SD, n=5]. Peripheral blood [PB] mononuclear cells showed 3-fold less protection. Adding 125,000 CBMC to the medium below the membrane instead of directly to slices reduced brain cell death 60 ± 8 % [mean +/-SD; n=3], suggesting that CBMC produce diffusible protective factors. To identify what types of cells mediate protection, we immunomagnetically depleted specific cell types and added depleted CBMC populations to OGD shocked cultures. Depleting CD14+ cells reduced the protective activity of CBC 3.5-fold, but depleting CD3+, CD19+, or CD34+ cells did not remove protective activity. Positively selected CD14+ also protected brain cultures from OGD, but CD3+ and CD19+ enriched populations did not. CD14+ cells selected from PB were 4-fold less active than CD14+ cells isolated from CB. Thus, CD14+ cells from CB are uniquely active in protecting brain cells from OGD induced death. We are now exploring how CB CD14+ cells interact with brain glia and neurons in these cultures and the molecular mechanisms by which CD14+ cells protect brain cells.

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HUMAN PLURIPOTENT STEM CELLS AMELIORATE NMDA-INDUCED HIPPOCAMPAL DEGENERATION AND RELATED FUNCTIONAL DEFICITS

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Seizures, trauma and many neurologic diseases induce damage to the CA3 region of hippocampus, resulting in extensive deficits in spatial navigation, memory consolidation, and depressive-type behaviors. Current drug treatments have limited effectiveness in addressing these memory problems. To attempt to use regenerative medicine to ameliorate these deficits, we used Celavie's human fetal, brain-derived, pluripotent, nontumorigenic, hypoimmunogenic stem cell line with a normal karyotype (hFSC). These stem cells have previously shown an ability to migrate, differentiate and reduce structural and functional deficits in other neurodegenerative models. We determined if hFSCs injected into an NMDA-lesioned hippocampus survive and possibly differentiate into mature functional neurons, thereby diminishing any behavioral and neuronal deficits. We induced hippocampal degeneration by stereotactically lesioning the CA3 regions bilaterally with the neurotoxin NMDA in 50 day old male Wistar rats (1 μl containing 7.5 mg/ml; -3.5 mm AP; ±2.0 L and -2.5 V). At 54 days of age, live hFSC, frozen-killed hFSC or HEK293T cells (500,000 cells of each type in 5 μl of cell suspension media), or cell suspension media (5 μl) were bilaterally implanted directly into the NMDA damaged area. The rats' spatial memory was tested two weeks later (68 days) with the Morris water-maze task, and novel and place-object tests. Our results confirmed that rats receiving live hFSC implantation performed significantly ($p<0.005$) better in the water maze task than any of the control groups. Novel and place object assays showed no significant differences among all the treatment groups. Immunohistochemistry results confirmed the survival of implanted hFSCs up to 28 days post-implantation in the rat CA3 region. Our study has shown that hFSC were able to survive in vivo and improve hippocampal functionality, highlighting the potential promise for stem cell treatment of brain damage in neurodegenerative diseases.

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NEURONAL AND GLIAL CELL COMPOSITION IN A MOUSE BRAIN SLICE CULTURE MODEL IS USEFUL IN DEVELOPING HUMAN CORD BLOOD DERIVED CELLULAR THERAPIES FOR NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY

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Our laboratory is developing novel cord blood (CB)-derived cellular therapies for patients with neuronal damage resulting from hypoxia-ischemia. To understand how CB cells mediate response to injury, we adapted and characterized the organotypic mouse brain slice culture model. The cellular composition of these brain slice cultures can be used to better understand the mechanisms underlying hypoxic injury and beneficial effects of cell therapies. We used immunofluorescence and image analysis to enumerate glial and neuronal cells in C57BL/6J mice-derived brain slices cultured on a semi-permeable membrane for 21 days in serum free medium. We compared the cellular composition of ex vivo brain slices to that of neonatal mouse brains. Selected brain slice cultures or brain sections were fixed in 4% paraformaldyde on ex-vivo culture or postnatal days 1, 3, 6, 9, 12, 15 and 21. Immunohistochemical staining differentiated astrocytes (GFAP), neurons (NeuN), oligodendrocytes (olig2) and microglia (Iba1) in cultured brain slices. Contiguous images of the periventricular regions were analyzed using fluorescence confocal microscopy. In brain slice cultures, neurons comprised approximately 40% (SD +/- 7%) of the total cell population, while glial cells made up 60% (SD +/- 3.5%) throughout the 21 day culture period. Conversely, in the age-matched neonatal brain sections, neurons maintained an average of 60% (SD +/- 6%) of the cellular composition, and remaining glial cells were 40% (SD +/- 4%). Both in vivo and in vitro, the relative proportions of the three glial cell populations were the same. These results establish concordance between the in vivo and ex vivo systems and validate the ex vivo brain slice model for use to further investigate effects of hypoxic injury. We have used this model system to investigate the protective effects of cord blood mononuclear cells after acute hypoxic injury on different types of brain cells.

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DUOC-01, A CANDIDATE CELL THERAPY PRODUCT DERIVED FROM BANKED CORD BLOOD, ACCELERATES BRAIN REMYELINATION IN NSG MICE FOLLOWING CUPRIZONE FEEDING

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We are developing a candidate cell therapy product, DUOC-01, derived from banked cord blood for use in the treatment of CNS demyelination. We have adapted the cuprizone model of reversible brain demyelination to