Conclusion: The present systematic review suggests that stem cell-based therapies for patients with stroke are effective and safe. However, properly designed randomized controlled trials are required.

214

ADIPOSE MESENCHYMAL STROMAL CELLS (AMSC) DIFFERENTIATE INTO NEURAL PROGENITOR CELLS (NPC) AFTER 24 HOURS OF CO CULTURE WITH EFFECTER CELLS (EC) AGAINST CENTRAL NERVOUS SYSTEM (CNS) PROTEINS

N Blasetti, LA Ugartemendia, GA Moviglia
CIITT, Universidad Maimonides, Buenos Aires, Argentina

Introduction: In 2006 (Cytotherapy 2006, 8:196-201) was reported that BM MSC co cultured with anti-CNS EC differentiate into NPC. To prove that aMSC share the same property and this process may be conducted under GMP rules we performed the following experiment.

Method: Adipose tissue obtained by lipectomy and dissociated with collagenase 4 in a GMP facility. aMSC were isolated by attachment and cultured for a week. EC were obtained from peripheral blood mononuclear cells, concentrated, were activated and expanded culturing them in DEMEM + human recombinant Insulin (Humalin), and 1% of Cerebrolysin . After 96 hours cells were harvested, washed and marked with anti CD8, CD56 and CD25. and negative selected with Clinimacs. EC and aMSC were co cultured for 24 hr to 120 hours. To test the aMSC differentiation into NPC cells were immune-fluorescent stained with anti nestin, tubulin 3, neu66, GFAP and Sox2 and MBP. and analyzed using confocal microscopy and FACS analysis.

Results: After 24 hours most of aMSC showed positive stain to nestin . At 48, 96 and 120 hours free cells and neurosphere structures showed positive stain for the rest of cell markers. In the neurosphere was able to distinguish cells positive for neu66, GFAP and sox2 proving multiple linage differentiation of these structures. No microbial contamination, persistence of lymphocytes or immune magnetic microbeads was detected in the harvested NPC cells.

Conclusions: aMSC may differentiate into NPC done from an adult individual without use of any cytokine, neurotropin, gene transpher, in a GMP facility.

215

TISSUE DISTRIBUTION OF A CORD BLOOD-DERIVED CELL PRODUCT FOLLOWING INTRATHECAL TRANSPLANTATION

R Storms[1], C Liu[2], T Gentry[1], J Zhou[1], A Ozamiz[1], B Rusche[1], A Balber[1], J Kurtzberg[1]
[1]Robertson Clinical and Translational Cell Therapy Program, Duke Translational Medicine Institute, Durham, North Carolina, United States, [2]Department of Surgery, Duke University, Durham, North Carolina, United States

We have developed an umbilical cord blood-derived cell product, DUOC-01, as a potential adjunct therapy for patients with certain inherited leukodystrophies. In clinical practice, DUOC-01 cells will be transplanted only after systemic cord blood transplantation and engraftment. Importantly, the DUOC-01 cells will be derived from the same cord blood unit that is to be used for the systemic transplant. To facilitate neural repair, the DUOC-01 cells will be delivered by intrathecal injection. The goals of this study were to determine the tissue distribution of DUOC-01 cells following their intrathecal injection, and to determine how long they remain detectable *in vivo*. Five different DUOC-01 cell preparations were cultured per GMP-compliant Standard Operating Procedures. For each transplant, 10^5 cells were delivered by intrathecal injection into neonatal ($\leq$ 2 days old) NOD/SCID-IL2Rγ^{null} mice. After periods of up to 56 days post-transplantation, the mice were sacrificed to analyze six tissues (brain, spinal cord, lungs, liver, spleen and bone marrow) for their content of human cells, as determined using quantitative PCR to detect human Alu DNA sequences. Within the first 24 hours post-transplantation, the DUOC-01 cells were detected within multiple tissues in 5 of 5 mice. These included the brain, spinal cord, lungs or, to a lesser degree, the liver. Human cells remained detectable within 11 of 20 mice that were analyzed between 7 and 56 days post-transplantation. However, at these later time points, the cells were detected only within the brain and spinal cord. Furthermore, in 8 mice that had human cells detectable within both the brain and spinal cord, the human DNA was more prevalent in the brain. These studies indicate that immediately following intrathecal injection the DUOC-01 cells distribute to both neural and non-neural tissues. However, in the long-term, the cells only remained detectable in the neural spaces encompassed by the brain and spinal cord.

216

ANGIOGENIC PERFORMANCE OF MAGNETIZED ENDOTHELIAL PROGENITOR CELLS FOR STROKE THERAPIES

A Rosell[1], E Carenza[2], V Barceló[1], A Morancho[1], J Montaner[1], A Roig[2]
[1]Neurovascular Research Laboratory, Vall d'Hebron Research Institute, Barcelona, Spain, [2]Nanoaprticles and Nanocomposites Group, 2Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Cerdanyola, Spain

IEndothelial Progenitor Cells (EPCs) are good candidates for cell-based therapies to treat ischemic diseases by inducing angiogenesis. We propose that EPCs can be magnetized with iron oxide superparamagnetic nanoparticles (SPIONs) and guide them into the ischemic brain with an external magnetic device to enhance neurorepair. SPIONs were synthesized by the thermal decomposition. EPCs were co-incubated for 24 hours and cell viability and cell function were assessed (MTT, tubulogenesis, migration and growth factors secretion). Cell magnetization was verified by SQUID magnetometry and transmission electron microscopy (TEM). A Magnetic Resonance (MR) study was conducted to determine the relaxometric properties of SPIONs and magnetized EPCs. In vivo, magnetized EPCs were guided to specific cortical areas with an magnet device after intravenous administration and brain angiogenesis was measured in a mouse model of cerebral ischemia. EPCs were successfully magnetized by SPIONs without affecting cell viability. TEM showed SPIONs stored in cytoplasmatic endosomes/lysosomes and EPCs were tracked in T2 weighted images by MRI. Magnetized EPCs were fully functional and the secretion of important growth factors was enhanced compared to non-magnetized EPCs. In this regard a proteome array showed a fold change >2 in magnetized vs. control cells for FGF, PDGF-BB, PD-ECGF or IGFBP-3, among others. MR images showed that brain tissue under the influence of magnetic forces accumulated hypointense signals consistent with magnetized EPCs engraftment after intravenous administration. Finally, in a mouse model of ischemia magnetized EPCs were intravenously administered and vessel density was found increased in specific cortical areas of animals with implanted magnetic devices. We show that EPCs magnetization with SPIONs might be a powerful tool for precise cell guidance and to potentiate their angiogenic properties through growth factors' secretion in the context of ischemia.

217

NEURONAL PROGRAMMING OF BONE MARROW MESENCHYMAL STEM CELLS

E André[1,2,3], P Resnier[1,2], L Sindji[1,2], AP Lopez[3], P Schiller[4], C Passirani[1,2], B Seijo[3], A Sanchez[3], C Montero-Menei[1,2]
[1]Micro et Nanomédecines Biomimétiques, inserm U1066, Angers, maine et loire, France, [2]PRES LUNAM , University of Angers, Angers, maine et loire, France, [3]Pharmacy and Pharmaceutical Technology, University of Pharmacy, santiago de compostela, La coruna, Spain, [4]Biochemistry & Molecular Biology, Miami Miller School of Medicine, Miami, Florida, United States

Hungtington's disease (HD) is an autosomal dominant genetic disease, associated with the progressive loss of the GABAergic neurons in the striatum. Among the strategies to cure this disease, is cellular transplantation to restore lost cells. Clinical studies with foetal GABAergic precursors have shown promising results, but problems with availability and ethical issues limit their use. Mesenchymal stem cells (MSC) are an interesting source of cells for brain regenerative medicine and more particularly the "Marrow-Isolated Adult Multilineage Inducible" (MIAMI) cells a homogeneous subpopulation, which express several pluripotency markers (Oct4, Sox2, Nanog), may differentiate toward a neuronal phenotype and secrete tissue repair factors. In order to enhance their neuronal differentiation, we chose to use RNA interference (siRNA) against the neuronal inhibitory factor REST to commit them toward a neural/neuronal phenotype(3). Non-viral nanoparticle-based vectors, which have an efficient transfection level, associated to low toxicity, have been developed for this neuronal programming purpose. Two different novel systems have been formulated for neuronal programming: lipid nanocapsules (LNC) and nanoparticles based on sorbitan esters (Span). Adapting the method by Heurtault et al, we optimized basic lipid nanocapsules (LNC) associating siRNA (size: 95nm and ζ potential : +10mV). On the other hand, we developed nanoparticles based on sorbitan esters (Span) and pullulan associating siRNA (size: 200 nm, ζ potential: - 43 mV). Both systems provide an efficient siRNA association (around 50%), showing appropriate stability during storage and a good efficiency of transfection. Indeed, forty-eight hours after the transfection, the siRNA seem to induce neuronal differentiation. Therefore we can conclude that LNC and Span nanosystems seem to be two promising systems with appropriate characteristics for siRNA transfection and cell therapy.