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Poster Presentation

Combined Treatment of Spinal Cord Injury Using Transplantation of Motoneurons Derived Adipose Stem Cells and Adipose Mesenchymal Stem Cells Transfected with GDNF Following Valproic Acid Treatment

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Abstract

According to the National Spinal Cord Injury (SCI) Statistic Center, 41.3% of SCIs occur as a result of motor vehicle crashes or motorcycle accidents. SCI is a devastating condition that effects on motor and sensory system. There is no definitive treatment for SCI. Therefore recent studies enhanced approach treatment in order to augmentation of locomotors rating scales. Currently, development in neurotransplantation and gene transfer techniques has introduced a spectrum of promising strategies for treatment of SCI. In this study, we used combined treatment of SCI using transplantation of motoneurons derived from adipose stem cells and adipose mesenchymal stem cell (MSC) transfected with GDNF following Valproic acid treatment. Adipose tissue isolation of perinephric fat in rat and then was digested with collagenase, followed by filter and centrifugation. The isolated adipose stromal cells were cultured and then MSC markers were evaluated by RT-PCR and immunocytochemistry; 2 to 5 passage cells were used for ex vivo gene delivery. Then adipose derived stem cells (ADSCs) derived into neurospheres which evaluated by immunocystochemistry and RT-PCR assay. The expression of islet-1, oligo-2 and HLXB9 in induced motoneuron like cells (MNLCs) from neurospheres evaluated by RT-PCR and immunocytochemistry. To identify the funsctional MNLCs, a co-culture preparation of MNLCs and myocytes, Calcium ion imaging and synaptic vesicle release were used. ADSCs treated with a mixture of preinducer (B27,EGF and bFGF) and inducers factors(Shh and RA) adopted a morphology similar to MNLCs. Other group of ADSCs was transfect by vector pLVPT-GDNF-trTR-KRAB-2SM2 and then the two group's cell transplant to contiusion model of rat SCI that administrate Valproic acid treatment. Immunocytochemical staining and RT-PCR approved that the treated cells expressed the motoneuron markers islet-1; oligo-2 and HLXB9.The co-cultured with myocytes indicate the formation of neuromuscular connections between MNLCs and myocytes. After two week, MNLCs showed high HLXB9 expression, indicative of full differentiation. Also, the release rate of synaptic vesicles using FM1-43 in the induced MNLCs was 10 fold. Moreover a calcium imaging with fluo-4 results approved that functional excitatory synaptic connections can influence the activity of MNLCs. Result of real time RT-PCR and SDS page and western blotting technique showed that transfected cells secrete human GDNF at high level. Basso-Beattie-Bresnahan (BBB) test scores of spinal cord injured rats treated with adipose MSC transfected with GDNF+MNLCs at 7 days+VPA (300g/kg and 12h post injury) were significantly improved as compared to scores of rats similarly injured (P<0.05). The H/M ratio decreased following the treated with adipose MSC transfected with GDNF+MNLCs (P<0.05). The cavity volume in this group was significantly reduced compared with the control (saline-injected) group (P<0.05). The level of GFAP was significantly decreased in this group, while it was significantly increase in the control rats (P<0.05). These findings indicate that neurotransplantation and gene transfer techniques can be used in clinical applications and treatment of CNS disorders.

Keywords: Motoneuron, Valproic Acid, B27, Retinoic Acid.

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