CONTRIBUTION OF DONOR DERIVED BONE MARROW STROMAL CELLS AND NEUROTROPHIC FACTOR FOR REGENERATION OF PERIPHERAL NERVE DEFECTS

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Introduction Reconstitution of peripheral nerve injury by nerve transplantation is one of the methods for reconstruction of long nerve defect. Supportive therapy with bone marrow stromal cells (BMSCs) has shown enhancement of nerve regeneration. This study was performed to asses the effect of BMSCs in nerve gaps repaired with isogenic epineural tubes filled with isogenic and allogenic BMSCs. Possible contribution of neurotrophic factors such as nerve growth factor (NGF) and Laminin B2 for nerve regeneration was assessed.
Methods Total of 54 isogenic epineural tubes were transplanted in 3 experimental groups (18 animals each). Group 1 control saline, Group 2 isogenic BMSCs (Lewis ($RT1^l$)) and Group 3 allogenic BMSCs (ACI ($RT1^a$)). Transplantation in Group 2 and 3 was supported with BMSCs therapy ($2x10^6$) delivered directly into transplanted epineural tube. Before transplantation BMSCs were stained with PKH-dye to assess migratory potential and ability for neural differentiation. Evaluation at 6, 12 and 24 weeks post-transplant included Gastrocnemius Muscle Index (GMI), sensory and motor recovery was evaluated by pinprick, toe-spread and Somato-Sensory Evoked Potentials (SSEP). Toluidin blue staining determined number of regenerated axons. Immunostaining with NGF and Laminin B2 assessed the expression of neurotrophic factors and regenerative potential of transplanted BMSCs within the epineural tubes.
Results Functional assessment by pin prick test, 6 weeks after transplantation, showed in all groups score 3. Toe spread for groups 1, 2 and 3 was respectively 1.7; 2; 1. SSEP in groups 1, 2 and 3 (P1, N2-latencies; P1, N2 % of normal values) was respectively (20.2; 23.6; 113; 95), (17.5; 18.1; 98; 73) and (15.7; 21.65; 88; 87). GMI in groups 1,2 and 3 respectively (0.45; 0.48; 0.47). Histology revealed first signs of axonal regeneration in all groups at 6 weeks. Group 2 showed higher number per measured field of regenerated axons (90.6 ± 26.9) compared to Group 1 (71.4 ± 3.0) and 3 (76.4 ± 5.4). In group 2 and 3 (with BMSCs) PKH positive cells were found in proximal part of transplanted tube. Immunostaining with NGF confirmed upregulation of NGF in proximal segment of tube compared to middle and distal parts. Moreover, NGF-staining in combination with PKH-staining confirmed that BMSCs differentiated into neural tissue. Differentiation efficacy was greater after transplantation in isogenic (Lewis) BMSCs compared to allogenic (ACI) BMSCs. NGF upregulation in groups 2 and 3 correlated with upregulation of Laminin B2 in both groups, indicating active nerve regeneration.
Conclusion In this study co-transplantation of BMSCs with epineural tube enhanced regeneration of peripheral nerve defects, confirmed by increased expression of neurotrophic factors such as NGF and Laminin B2. Better functional recovery and axonal regeneration was seen in epineural tube grafts supported with BMSCs compared to saline control group. Finally, we have proven regenerative potential of BMSCs through their differentiation into the neural tissue.