Therapeutic Effect of Gene Modified Bone Marrow Hemopoeitic Stem Cells (BMHSCs) Versus Unmodified BMHSCs Derived Exosomes in Early Diabetic Retinopathy Rat Model

Review Article

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ABSTRACT

The present study aimed at investigating and comparing the therapeutic effect of RPE65 and CYP46a1 gene modified bone marrow hemopoietic stem cells (BMHSCs) versus unmodified BMHSCs derived exosomes (BMHSCs-EXOs) in streptozotocin (STZ) induced early diabetic retinopathy (DR) in male albino rat model.

This study was conducted on 32 adult male albino rats with average body weight 200 grams, that were divided into: Donor Group: of 4 rats, 2 were used for BMHSCs-EXOs isolation, culture, phenotyping and labeling, the retina of the other 2 rats were homogenized and processed for RPE65 and CYP46a1 gene preparation by using reverse transcriptase. Lipofectamine 3000 transfection was performed. Group I (Control Group): included 6 rats. Group II (DR Group): included 8 rats. Diabetes was induced by a single IP injection of STZ at a dose of 50 mg/kg dissolved in 0.5 ml citrate buffer for each rat. 3 days following STZ injection, diabetes was confirmed by measuring blood glucose level. The animals were considered diabetic if their blood glucose level was higher than 200 mg/dl. Group III (DR, BMHSCs-EXOs treated Group): included 7 rats. 2 weeks following confirmation of diabetes, 200µg of cultured and PKH-26 labeled rat BMHSCs-EXOs, suspended in 0.5ml PBS were injected in four prepared doses (once per week) IP. Group IV (DR, Gene modified BMHSCs treated Group): included 7 rats. 2 weeks following confirmation, 1x106 of cultured and green fluorescent protein (GFP) labeled, 1µg RPE65 and 1µg CYP46a1 transfected rat BMHSCs suspended in 1 ml PBS were injected once IP. All rats were sacrificed 6 weeks, following the day of confirmation of diabetes. In Vitro Studies and animal studies including electroretinogram (ERG), serological, lipid peroxidation (LPO) and glutathione peroxidase (GPx) assessment, histological, caspase3 and vascular endothelial growth factor (VEGF) markers, morphometric, fluorescent microscopic and statistical studies were performed. Quantitation of RPE65 and CYP46a1 genes, and RPE65 and CYP46A1proteins was done.

In group II, mean amplitudes of a and b waves in ERG denoted a sig increase and decrease respectively, mean values of blood glucose indicated a sig increase, mean LPO and GPx values indicated a sig increase and decrease respectively. Apparently thickened retina, disruptions, vacuolations, separations and dark nuclei in various layers, detached RPE, congested capillaries, disfiguring, loss of architecture and disorganization were seen. Group III revealed less vacuolations and dark nuclei. Group IV clarified few vacuolations and dark nuclei. Group III expressed less extensive and group IV focal caspase3 IE versus group II. Group III expressed less numerous and group IV localized VEGF IE versus group II. Morphological and immunohistochemical data were confirmed morphometrically. In group III, PKH26 labeled BMHSCs-EXOs were detected and in group IV GFP labeled gene modified BMHSCs were found. The mean RPE65 and CYP46a1 gene values, and RPE65 and CYP46A1 protein values indicated a sig decrease in group II versus all groups, in group III versus groups I and IV.

Conclusion: The induced morphological and morphometric changes in the retina of rat by early DR that were confirmed by corresponding electrophysiological, biochemical, PCR and Wb values changes were improved to a great extent by treatment with RPE65 and CYP46a1 gene modified BMHSCs. Treatment by repeated injections of BMHSCs-EXOs proved less remarkable effect.

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