Rat Bone Marrow Mesenchymal Stem Cell Differentiation to Insulin Producing Cells and Evaluation their Responses in Vitro and in Vivo
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Background
In recent years, many researchers have attempted to cure diabetes by using stem cells technology. Stem cells from different sources have capability to differentiate into insulin producing cells (IPCs) by different methods. The obstacles of these methods are their expensive materials and complexity of method which are practically disadvantages for producing enough transplantable IPCs that can cure a diabetic patient. The aim of present study was to test a method for isolation and differentiation of rat bone marrow mesenchymal stem cells (BMSC) into IPCs which is simple method and produce abundant IPCs.

Methods:
Adult male Wistar rats 4-6 weeks age and 200-250 g weight were used. Bone marrow was isolated from femoral bone under aseptic condition and cultured as monolayer in DMEM in a density of 1x10^6/well at 37°C and 5% CO₂ for 72 h. After three passages, the cells were differentiated into adiposities and osteocyte cells which approved using oil red and alizarin red staining, respectively. To produce IPCs, BMSC was cultured in low glucose DMEM, with 1% DMSO and without FBS for three days on collagenated coverslips. After three days, the culture medium was changed to DMEM containing 25 mM glucose plus 10% FBS and incubated for 7 days. For assessment of insulin secretion capability of IPCs, the cells were incubated in DMEM containing 5.5 or 25 mM glucose with or without IBMX for two hours. Samples from the medium were taken for subsequent insulin assay. For in vivo assay, the mice were made diabetic by injection 200 mg/kg streptozocin intraperitoneally, which produced a fasting blood sugar about 450 mg/dl. For each mouse, 10⁶ IPCs were injected intraperitoneally. Control mice were injected with 100 µl normal saline. The mice blood sugars were checked every 3 days with a glucometer device.

Results:
The content of secreted insulin in 5.5 mM glucose concentration was about 40 miu/ml that raised to 72 miu/ml with incubation with 25 mM glucose. The insulin secretion was potentiated by IBMX which increased to 104 miu/ml. One week after IPC transplantation to diabetic mice, fasting blood sugar was started to decrease. After two weeks of transplantation, the blood glucose was fallen to 265 mg/dL comparing to control mice which their fasting blood sugar was about 450 mg/dL until end of study.

Conclusion:
In this study we introduced a simple, valuable and low cost method to produce insulin producing cells from bone marrow mesenchymal stem cells which could be translated into human clinical trials.

Keywords: Mesenchymal stem cells, Transplantation, Diabetes.

Poster Presentation
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