Rat Bone Marrow Mesenchymal Stem Cell Differentiation to Insulin Producing Cells and Evaluation their Responses in Vitro and in Vivo

*Seyyed Abbas Zojaj [1], Reza Shafiee-Nick[1], Ahmad Ghorbani[1]

[1]Department of Pharmacology, Pharmacological Research Center of Medicinal Plants, Faculty of Medicine, Mashhad University of Medical Sciences Mashhad, Iran.

Background

In recent years, many researchers haveattempted to cure diabetes by using stem cells technology. Stem cells from different sources have capabilityto differentiateinto insulin producing cells (IPCs) by different methods. The obstaclesof these methods aretheirexpensive materials and complexity ofmethodswhichare practicallydisadvantagesfor producing enough transplantableIPCs 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 asepticconditionand cultured as monolayerin DMEM in a density of 1×10^6/well at 37ºC and 5% CO_2 for72 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, theculture medium was changedto DMEM containing 25 mM glucose plus10%FBS and incubated for 7 days.

For assessment of insulin secretion capabilityof IPCs, the cells were incubated in DMEM containing5.5 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/kgstreptozocinintraperitoneally,which produced a fasting blood sugar about 450 mg/dl. For each mouse, 10^5 IPCs were injectedintraperitonally. Control mice were injected with 100 µl normal saline.The mice blood sugars were checked every 3 days with a glocoumeter device.

Results:

The content of secreted insulin in 5.5 m M glucose concentration was about 40miu/ml that raised to72 miu/ml with incubation with 25 mM glucose. The insulin secretion was potentiated by IBMX which increased to 104miu/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

***Corresponding Author:** Seyyed Abbas Zojaj, Department of Pharmacology, Pharmacological Research Center of Medicinal Plants, Faculty of Medicine, Mashhad University of Medical Sciences Mashhad, Iran.