Co-Culture of Neonate Mouse Spermatogonial Stem Cells with Sertoli Cells: Inductive Role of Melatonin following Transplantation: Adult Azoospermia Mouse Model

M. Abbasi, S. Navid, M. Pour Ahmadi, M. Majidi Zolbin

***Abstract*—We have recently reported that melatonin as antioxidant enhances the efficacy of colonization of spermatogonial stem cells (SSCs). Melatonin as an antioxidant plays a vital role in development of SSCs in vitro. This study aimed to investigate evaluation of sertoli cells and melatonin simultaneously on SSC proliferation following transplantation to testis of adult mouse busulfan-treated azoospermia model. SSCs and sertoli cells were isolated from the testes of three to six day old male mice.To determine the purity, Flow cytometry technique using PLZF antibody were evaluated. Isolated testicular cells were cultured in αMEM medium in the absence (control group) or presence (experimental group) of sertoli cells and melatonin extract for 2 weeks. We then transplanted SSCs by injection into the azoospermia mice model. Higher viability, proliferation, and Id4, Plzf, expression were observed in the presence of simultaneous sertoli cells and melatonin in vitro. Moreover, immunocytochemistry results showed higher Oct4 expression in this group. Eight week after transplantation, injected cells were localized at the base of seminiferous tubules in the recipient testes. The number of spermatogonia and the weight of testis were higher in experimental group relative to control group. The results of our study suggest that this new protocol can increase the transplantation of these cells can be useful in treatment of male infertility.**

***Keywords*—Melatonin, spermatogonial stem cell, colonization, transplantation, mouse.**

I. Introduction

SSCs are the source of spermatogenesis and fertility in men. The number of these stem cells, like tissue-specific stem cells, is very limited and was counted about 0.03 percent of all stem cells in rodent testes. The treatment of cancer with chemotherapy and radiation can cause a high percentage of male infertility [1], [2]. One of the ways to treat infertility is to culture of SSCs in vitro. Sertoli cell plays a critical role in the maintenance of SSCs both in vivo and in vitro [3]. Recent studies have paid much attention to growth factors such as leukemia inhibitory factor (LIF), glia cell line derived neurotrophic factor (GDNF)in vitro proliferation of SSCs [4]-[6]. Melatonin (N-acetyl-5-methoxytryptamine) has many different roles, such as regulation of immune response and cell signaling, protection of fatty acids from oxidation and on costatic actions, and antiapoptotic and anti-aging properties on many cells [7], [8]. The effects of melatonin supplementation on the culture and co-culture of SSCs with sertoli cells as well as transplantation of SSCs were evaluated in this study. Our finding showed that sertoli cells and melatonin simultaneously on SSC proliferation can increase transplantation of SSCs.

M.Abbasi. is with the Anatomy Department Tehran University of Medical Science, Tehran, Iran abbasima@sina.tums.ac.ir
S. Navid is with Anatomy Department Tehran University of Medical Science, Tehran, Iran. navideshadan@yahoo.com.
M. Pourahmadi Research Center for Noncomunicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran
M. Majidi Zolbin is with the Anatomy Department, Tehran University of Medical Science, Tehran, Iran, m-majidyzolbin@razi.tums.ac.ir

II. Materials and Methods

A. Culture of SSCs

Neonatal mice between three- to 6-day-old NMRI (National Medical Research Institute) male mice were maintained under standard conditions. Animal experiments in this study were considered by the ethics committee of Tehran University of Medical Sciences in accordance with the university's guidelines. Testicular cells were isolated by two-step enzymatic digestion. Testes were first collected and weighed as well astunica albuginea were removed. Testes were then cut into small pieces and transferred to a digestion solution containing collagenase type IV (1 mg/mL, Sigma), DNase (10 µg/ml, Sigma), and hyaluronidase (0.5 mg/ mL, Sigma) for 20 min at 37°C in a 5% CO2 incubator. The first step of enzymatic digestion was dispersed the tubules and the second step of enzymatic digestion was dispersed the isolated cells and were then washed with phosphate buffered saline (PBS; Sigma, Germany).A flow cytometry technique was carried out on testicular cells using PLZFto assess the percentage of purity of SSCs.

SSCs with sertoli cells were cultured for two weeks (density of 2×105 cells/ cm2) in the MEMα containing 10% fetal bovine serum (FBS; Sigma), 1 x nonessential amino acids (Invitrogen, USA), 0.1 mM 2-mercaptoethanol (Sigma), 103U/ml human recombinant leukemia inhibitory factor (LIF; B&D, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (both from Sigma). We added 10 ng/ml Glial cell line-derived neurotrophic factor to each culture dish (GDNF; R&D, USA). In the treatment group, 100 µM of melatonin (Sigma) was added to the basic medium culture [9], [10].The culture dishes were maintained at 32°C in an atmosphere humidified with 5% CO2. The cells were then passaged with trypsin/EDTA solution two time during the period of culture.