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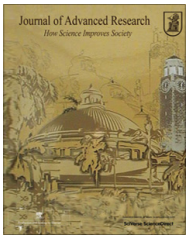

ORIGINAL ARTICLE

Therapeutic efficacy of amniotic membrane stem cells and adipose tissue stem cells in rats with chemically induced ovarian failure

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ABSTRACT

The present study was conducted to compare between the therapeutic efficacies of human amniotic membrane-derived stem cells (hAM-MSCs) vs. adipose tissue derived stem cells (AD-MSCs) in cyclophosphamide (CTX)-induced ovarian failure in rats. Forty-eight adult female rats were included in the study; 10 rats were used as control group. Thirty-eight rats were injected with CTX to induce ovarian failure and divided into four groups: ovarian failure (IOF) (IOF group), IOF + phosphate buffer saline (PBS group), IOF + hAM-MSCs group and IOF + AD-MSCs group. Serum levels of FSH and estradiol (E2) were assessed. Histopathological examination of the ovarian tissues was performed and quantitative gene expressions of *Oct-4*, *Stra8* and *integrin beta-1* genes were conducted by quantitative real time PCR. Results showed that IOF and IOF + PBS rat groups exhibited decreased ovarian follicles, increased interstitial fibrosis with significant decrease of serum E2, significant increase serum FSH level and significant down-regulation of *Stra8* and *integrin beta-1*. In hAM-MSCs and AD-MSCs rat groups, there were increased follicles and corpora with evident the presence of oocytes, significant increase in serum E2, significant decrease in serum FSH levels (in hAM-MSCs treated group only) and significant up-regulation of the three studied genes with higher levels in hAM-MSCs treated rats group when compared to AD-MSCs treated rats group. In Conclusion, administration of either hAM-derived MSCs or AD-MSCs exerts a significant therapeutic efficacy in chemotherapy induced ovarian insult in rats. hAM-MSCs exert higher therapeutic efficacy as compared to AD-MSCs.

Introduction

Premature ovarian failure (POF) or premature ovarian insufficiency (POI) is defined as cessation of menstrual periods (amenorrhea for 4 months or more), increased levels of FSH to menopausal level (usually over 40 IU L^{-1} obtained on 2 occasions at least 1 month apart), and diminished levels of

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estrogens (estradiol levels less than 50 pg mL^{-1}) before the age of 40 [1].

At present about 25% of all forms of POF can be classified as iatrogenic and related to cancer treatment [2] such as chemotherapy or radiotherapy. Also, POI may be caused by autoimmunity, genetic factors as Turner's syndrome, fragile X syndrome and inhibin alpha *(INHA)* gene affection. In addition, metabolic disorders such as classic galactosemia, virus infection such as HIV and mumps, toxins and lifestyle factors such as cigarette smoking are associated with the development of POI [3]. However, the majority of cases remain idiopathic.

Bone marrow derived MSCs (BM-MSCs) are the most common source of clinically used MSCs. However, adult BM has limitations; it needs invasive harvesting which is accompanied by a risk of infection. It yields a small number of cells. In addition, the number, differentiation potential, and life span of BM-MSCs decline with patient age [4].

Amniotic membrane (AM)-derived stem cells are isolated from amnion that is normally discarded. These cells are therefore readily available, and ethical concerns regarding the isolation of stem cells from the amniotic membrane are minimized [5]. In addition to that, amniotic stem cells have not been found to form teratomas when transplanted *in vivo* [6]. Furthermore, hAM-MSCs may be considered superior to adult MSCs in their proliferation and differentiation potential [6].

hAM-MSCs express pluripotency markers including *OCT4*, *SOX2*, and *NANOG* [7], high expansion *in vitro* and multilineage differentiation capacity potential into cells derived from the three germ layers [8]. However, at this time whether hAM-MSCs can restore ovarian function is unclear.

Oct-4 gene is required for primordial germ cell survival and loss of Oct4 function leads to apoptosis of primordial germ cells (PGCs) so it is essential for maintaining viability of mammalian germ line [9]. The discovery of the essential role of *Oct4* in induced pluripotent stem (iPS) cell technology further highlights its irreplaceable function in establishing pluripotency [10].

Integrins are transmembrane receptors that link the extracellular matrix (ECM) environment with intracellular signaling, thus regulating multiple cell functions such as cell survival, proliferation, migration, and differentiation. *CD29* (*integrin beta-1*) gene expression is believed to be crucial for tissue repair [11].

Stra8 is a gene that is specifically expressed in mammalian germ cells before their transition from mitosis to meiosis [12]. It is a regulator of meiotic initiation in females [13] so it is considered a meiotic inducer [14] and a pre-meiosis specific marker [15].

To address stem cells' therapeutic potential in ovarian failure we compared the therapeutic efficacy between isolated hAM-MSCs (cells from fetal tissues) and human ADMSCs (cells from adult tissues) in supporting follicular development, hormonal production by the ovary and expression of pluripotent markers, germ cells markers and anchoring receptors in experimental animal model of chemotherapy induced ovarian failure.

Material and methods

This study is a prospective case control animal study performed in the Faculty of Medicine, Cairo University, at the Unit of Biochemistry and Molecular Biology. The design of this work was divided into 2 steps: *in vitro* and *in vivo* study.

In vitro study

Isolation and propagation of hAM-MSCs and AD-MSCs from human source

Placentas were freshly collected postpartum from Gynecology and Obstetric Department, Faculty of Medicine Cairo University. The amniotic membranes were mechanically separated from placentas taken from normal full-term uncomplicated elective cesarean sections after obtaining a written informed consent. They were transported in cold phosphate buffer saline (PBS; Gibco/Invitrogen, Grand Island, New York, USA) solution in a thermally insulated container on ice. The amniotic membranes were processed within 1 h and subjected to collagenase II enzymatic digestion. Collagenase II solution was prepared at 100 U/mL in DMEM and 15 mL of collagenase II solution was added to the amniotic membranes and incubated for 2 h at 37 °C in 5% CO_2 atmosphere with occasional shaking. Digested amniotic membranes were filtered through a 40 μm cell strainer to remove large tissue aggregates.

After enzyme digestion and filtration, cells were washed in phosphate-buffered saline (PBS) (Cellgro, USA) and centrifuged at 350 g for 5 min. The cell pellet was resuspended in the basal culture medium, low glucose Dulbecco's Modified Eagle's Medium (LG-DMEM; Gibco BRL, USA) containing 10% FBS (Fetal bovine serum; Gibco BRL, USA). The cells were seeded into T75 polystyrene cell culture flasks (Nunc, USA) and incubated in a humidified atmosphere with 5% CO2. The basal culture medium was changed 3 times a week and passaged once the cells reached 80–90% confluency [16].

Human adipose tissue was collected by human abdominal subcutaneous adipose tissue aspiration during surgery after obtaining an informed medical consent. It was transported on ice insulated container. The adipose tissue was washed extensively with PBS to remove contaminating debris and red blood cells then minced with scissors and enzymatic digestion was performed as described with amniotic membranes with collagenase II in PBS for 60 min at 37 °C with gentle agitation. Digested tissue was filtered through a 200-μm mesh filter to remove debris and centrifuged at 600 g for 5 min to obtain cellular pellets. Erythrocytes were removed by treatment with erythrocyte lysis buffer. The cells were cultured as described for the cells isolated from amniotic membranes [17].

Labeling the MSCs to detect their homing into the ovarian tissue in the animal model

MSCs were labeled with PKH26 Red Fluorescent Cell Linker Kit (Sigma–Aldrich) and injected into the tail vein of rats. After one month of injection, the ovarian tissue was examined with a fluorescence microscope to detect the cells stained with PKH26 dye to ensure homing and trace the injected cells in the ovarian tissue.

Characterization of isolated human hAM-MSCs and AD-MSCs

Quantitative expression of mesenchymal stem cells, amniotic membrane and adipose tissue markers was conducted with using CYTOMICS FC 500 Flow Cytometer (Beckman coulter,