Cytotherapy, 2013; 15: 64–75

International Society for Cellular Therapy
ISCT®

Mechanistic action of mesenchymal stem cell injection in the treatment of chemically induced ovarian failure in rabbits

SOMIA H. ABD-ALLAH[1], SALLY M. SHALABY[1], HEBA F. PASHA[1], AMAL S. EL-SHAL[1], NERMIN RAAFAT[1], SHEREN M. SHABRAWY[1], HANAN A. AWAD[1], MONA G. AMER[2], MAHMOUD A. GHARIB[3], EMAN A. EL GENDY[3], AMAL A. RASLAN[4] & HASSAN M. EL-KELAWY[5]

[1]*Medical Biochemistry Department, Faculty of Medicine,* [2]*Histology & Cell Biology Department, Faculty of Medicine,* [3]*Obestetrics and Gynecology Department, Faculty of Medicine,* [4]*The Veterinary Clinic, Faculty of Veterinary Medicine, and* [5]*Animal Production Department, Institute of Efficient Production, Zagazig University, Zagazig, Egypt*

Abstract

Background. No curative treatment is known for primary ovarian failure; however, mesenchymal stem cells (MSCs), through self-renewal and regeneration, push the trial to evaluate their role in the treatment of ovarian failure. The aim of this study was to explore the impact of MSCs on cyclophosphamide (CTX)-induced ovarian failure in rabbits and to clarify the mechanism(s) by which MSCs exert their action. *Methods.* Thirty-five adult female rabbits were injected with CTX to induce ovarian failure. Five rabbits were euthanized after the last injection of CTX for histological examination. The others (30 rabbits) were further subdivided into two groups: group 1 (ovarian failure group, 15 rabbits) received no treatment; group 2 (ovarian failure and MSC recipient group, 15 rabbits) received MSCs isolated from extracted bone marrow of male rabbits. *Results.* A decrease of follicle-stimulating hormone and an increase of estrogen and vascular endothelial growth factor (VEGF) levels in the MSC recipient group versus the ovarian failure group were found. Weak caspase-3 expression and +ve proliferating cell nuclear antigen staining after MSC injection were detected. Cytological and histological examinations showed increased follicle numbers with apparent normal structure of ovarian follicles in the MSC recipient group. Moreover, Y chromosome–containing cells from male donors were present within the ovarian tissues in group 2. *Conclusions.* The current study suggests that intravenous injection of MSCs into rabbits with chemotherapy-induced ovarian damage improved ovarian function. MSCs accomplish this function by direct differentiation into specific cellular phenotypes and by secretion of VEGF, which influence the regeneration of the ovary.

Key Words: *caspase-3, cyclophosphamide, mesenchymal stem cells, ovarian failure, proliferating cell nuclear antigen, vascular endothelial growth factor*

Introduction

Premature ovarian failure is a syndrome characterized by lack of folliculogenesis and ovarian estrogen production, associated with amenorrhea and infertility in women under the age of 40 years (1). The syndrome is represented in 1% of menopausal women (2) and in 0.1% under the age of 30 years (3).

One of the most devastating consequences of cancer treatment in the young female population is ovarian damage, resulting in diminished fertility potential (4). Sterilization and early menopause in young female adults have a high-level impact on patient self-esteem and quality of life.

Mesenchymal stem cells (MSCs) residing within the bone marrow (BM) have the advantages of being easily accessible and readily available (5). Unlike embryonic stem cells, MSCs also have no controversies surrounding them such as ethical concerns, inflammatory response and tumorigenesis (6). However, BM-derived undifferentiated MSCs may be a source of carcinoma-associated fibroblasts, which may contribute to the promotion of tumor growth, invasiveness and metastasis; therefore it is thought that the clinical use of MSCs in the context of malignant conditions should be managed with extreme caution (7).

BM-derived MSCs have attracted considerable attention as tools for differentiation into several mesodermal lineages including osteoblasts, chondrocytes, adipocytes, cardiocytes, neural cells and

Correspondence: Dr **Somia H. Abd-Allah**, Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt. E-mail: dr.somiahassan@yahoo.com, sallyshalaby@hotmail.com

(Received 14 May 2012; accepted 16 August 2012)

http://dx.doi.org/10.1016/j.jcyt.2012.08.001

hematopoietic-supporting stroma and may therefore partly replace the impaired cells (8). One of the mechanisms of repair is the integration of MSCs into the tissue and replacement of damaged cells. Successful attempts of BM-derived MSC transplantation for repairing spinal, cardiac and cutaneous injuries, for instance, have been reported (9–11).

Vascular endothelial growth factor (VEGF) is produced by the majority of cell types. VEGF induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and plays a central role in the development of blood vessels in the thecal layer of follicles (12). It is widely believed that activation of caspase-3 leads to DNA fragmentation, a hallmark of apoptosis. The activation of caspase-3 was shown to decrease the number of oocytes and follicles (13). Proliferating cell nuclear antigen (PCNA) has also been suggested to be a key regulator during the development of ovarian follicles, but the exact function of PCNA in meiosis, particularly in primordial follicle formation, is unknown (14).

Currently, little is known about the therapeutic potential of BM-derived MSCs for chemotherapy-induced ovarian damage. Therefore, the aim of this study was to explore the therapeutic potency of intravenously administered BM-derived MSCs on ovarian function and structure for chemotherapy-induced ovarian failure in rabbits and to elucidate the role of VEGF as a mediator secreted by MSC, caspase-3 as an indicator of apoptotic event and PCNA as an essential regulator of the cell cycle after MSC therapy.

Methods

This study was performed in a stem cell research laboratory in the Medical Biochemistry & Molecular Biology Department in collaboration with the Histology & Cell Biology and Gynecology & Obstetrics departments, Faculty of Medicine, Zagazig University, Egypt.

Animal model

Healthy adult multiparous female Baladi black rabbits that weighed between 2,500 and 3,500 g were supplied from the Institute of Productive Efficiency, Zagazig University, Egypt, and used in the study. This study was conducted according to the Institute Review Board Instruction of Care and Use of Laboratory Animals. The age of the rabbits at the start of the study was 7–10 months. Rabbits were fed complete pellets and water under a controlled temperature (28–30°C) that is ideal for reproductive performance (15). To ensure adequate adaptation, they were observed in this environment for 7 days before commencing the experimental protocol. The rabbits did not show regular estrus cycles. They were considered to be reflex ovulators (always ovulating). To determine the estrus cycle in adult female rabbits, all of the rabbits were co-cycled by preparation of a vaginal smear. Vaginal smears were obtained 9–13 hours after induction of ovulation by mechanical stimulation of the vagina (16,17). The vaginal smear was taken with a cotton-tipped swab moistened with saline and inserted into vagina; epithelial cells were gently removed from the vaginal lumen and walls and transferred to a glass slide. Preparation of the smear and staining with papanicolaou stain was performed. Preparation of the smear and staining with papanicolaou stain was performed and examined microscopically. Three types of cells could be recognized: epithelial cells, cornified cells and leukocytes. Prominence of cornified cells indicated the estrous stage (18). Only rabbits in the estrous stage were included in the experiment. Five rabbits were euthanized for identification of normal histological structure of control rabbit ovary. Thirty-five rabbits were included in the study.

Experimental design

To establish animal models of chemotherapy-induced ovarian failure, 35 rabbits received a loading dose of intraperitoneal cyclophosphamide (CTX, 50 mg/kg) followed by daily intraperitoneal CTX injection of 8 mg/kg for 14 consecutive days, according to the protocol proposed by Desmeules and Devine (19). Five rabbits were euthanized after the last injection of CTX for histological examination. The others (30 rabbits) were further subdivided into two groups: group 1 (ovarian failure group, 15 rabbits) received no treatment; group 2 (ovarian failure and MSC recipient group, 15 rabbits) received MSC transplantation. Immediately after the last injection of CTX, MSCs (5×10^6 cell/200 g) were injected intravenously in the ear veins of the rabbits.

All rabbits of both groups were euthanized at the end of the study (6 weeks after CTX injection).

MSCs were prepared from extracted BM of Baladi black male rabbits, with age range from 2–3 months, weighing between 2 and 3 kg.

For estimation of follicle-stimulating hormone (FSH), estrogen (E2) and VEGF levels through the period of the study, five blood samples (3 mL each) were collected from the ear veins of the rabbits 9–13 h after mechanical vaginal stimulation. The five blood samples were the first before CTX injection, the second after the last CTX dose and the others at 2, 4 and 6 weeks after the last CTX injection. Serum