Evaluation of the effect of mesenchymal bone marrow stem cells injection on chemotherapy-induced ovarian failure in female albino rats

Thesis
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Presented by
Sarah Ali Abdelhameed Gouda
(M.B, B.CH)

Under supervision of

Prof. Dr. Moshira Abdelhakiim Rateb
Professor of Physiology
Faculty of Medicine - Cairo University

Prof. Dr. Hala Gabr Metwaly
Professor of Clinical Pathology
Faculty of Medicine - Cairo University

Dr. Hanan Ahmed Seddiq
Lecturer of Physiology
Faculty of Medicine - Cairo University

Faculty of Medicine
Cairo University
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Abstract

Objectives: This study aims at shedding light on possible answers for the following questions:

1-Can mesenchymal stem cells home in the ovaries of both control and chemotherapy treated animals. 2-Is there a possible role of TNF-α in homing of stem cells.

3-If they home, do they produce their effect through differentiation into follicles themselves or through secretion of some growth factors such as IGF-I.

Method: 72 female albino rats were randomly allocated into Control group & CTX group, each group was subdivided into 3 subgroups; Control group was subdivided into: Control A which did not receive any injection during, control B1 received Ip saline injections + single Iv saline, and control B2 received Ip saline injections + single Iv MSCs injection. CTX group was subdivided into: CTX A this subgroup received repeated Ip cyclophosphamide injections only, CTX B1 received Ip cyclophosphamide injections + single Iv saline injection and CTX B2 received cyclophosphamide Ip injections + single Iv MSCs injection. a further group (donor group) of 18 female rats of the same breed were used as MSCs donors.

The Experimental protocol was lasted for 12 weeks, divided into: Conditioning time period (1 week) followed by Injection-1 period which is lasted for 2 weeks during which the animals received Ip cyclophosphamide, saline injection or no injection at all according to their subgroups. Then, monitoring-1 period (M1) which took 1 week during which all animals were monitored for serum FSH, E2 levels and vaginal smears. At the end of (M1), 6 of animals in each subgroup were randomly sacrificed to test for ovarian histopathology and level of TNF-α in first sampling point. The rest of the animals in each subgroup were injected once with either stem cells or saline Iv or nothing according to their subgroups in Injection-2 point which was followed by monitoring-2 period (M2) lasted for 8 weeks, during which serum FSH and E2 were monitored twice at the end of the 2nd week of M2 and the end of the 8th weeks of M2. at the end of the experimental protocol, in second sampling point the remaining animals were sacrificed to evaluate ovarian histopathology & the expression of ovarian IGF-1.

Results:

Migration and homing of MSCs in the ovarian stroma of both control B2& CTX B2 with gradual partial Improvement of E2 and FSH levels as well as ovarian architecture and follicular number and maturation in CTX B2, elevation of ovarian TNF- α levels In CTX group and elevation of IGF-I immunohistochemical expressions in ovarian tissues of MSCs injected rats.

Conclusion:

Injected bone marrow derived MSCs can migrate and home in the stroma of the injured ovaries, with gradual partial improvement of hormonal function, follicular number, maturation and architecture of ovaries damaged by cyclophosphamide. IGF-I can play a role in such improvement after stem cells injection in chemotherapy- induced ovarian failure. Elevated ovarian TNF- α levels may have a role in migration and attraction of stem cells in vivo.

Key words: chemotherapy induced ovarian failure - cyclophosphamide - stem cells.

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List of abbreviations

ADSCs: Adipose-derived stem cells.

Akt: is a serine/threonine-specific protein kinase, also known as Protein Kinase B (PKB) that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration.

AMH: Anti-Müllerian hormone.

ASCs: Adult Stem Cells.

Bcl-2: B-cell lymphoma 2.

bFGF: Basic fibroblast growth factor .

BM: Bone marrow.

BMP-15: Bone morphogenetic protein-15.

BMSCs: Bone marrow- stem cells.

CAM: Cell adhesion molecules.

Caspases: Cysteine aspartate-specific proteases.

CCs: Cumulus cells.

CD: Cluster of differentiation.

CFU: Colony forming unit.

c-Kit: Mast/stem cell growth factor receptor (SCFR), also known as proto-oncogene c-Kit or tyrosine-protein kinase Kit or CD117, is a protein that in humans is encoded by the KIT gene.

CL: Corpus luteum.

COC: Cumulus–oocyte complex.

Cp: Conditioning period.

CTX: Chemotherapy.

DMEM: Dulbecco's Modified Eagle's Meduim.

DPSCs: Dental pulp stem cells.

E2: Estradiol.

ECM: Extracellular matrix.

EGF: Epidermal growth factor.

EGFR: Epidermal growth factor-receptor.

ELISA: Enzyme-linked immunosorbent assay.

EPIC: European Prospective Investigation into Cancer and Nutrition.

ESCs: Embryonic stem cells.

FACS: Fluorescence-activated cell sorting.

FGF: Fibroblast growth factor.

FSH: Follicle-stimulating hormone.

FSHR: Follicle-stimulating hormone receptor.

GDF-9: Growth Differentiation Factor-9.

GFP: Green fluorescent protein.

GnRH: Gonadotropin-releasing hormone.

GSs: Granulosa cells.

hEGCs: Human embryonic germ cells.

HGF: Hepatocyte growth factor.

HM: Homogenization medium

(HPG) axis: hypothalamic-pituitary-gonadal axis.

HSCs: hematopoietic stem cells.

ICT: International Society for Cellular Therapy

IGF-I: Insulin-like growth factor-I.

IGF-IR: Insulin-like growth factor-I receptor.

IL-6: Interleukin 6.

Ip: Intra-peritoneal.

iPs: Induced pluripotent stem cells.

Iv: Intravenous.

KL: Kit-ligand.

Klf4: Kruppel-like factor 4. .

LH: luteinizing hormone.

M1: Monitoring-1 period.

M2: Monitoring-2 period.

MGCs: Mural granulosa cells.

MSCs: Mesenchymal stem cells.

NanOg: (Land of youth in Irish language). It is a transcription factor in embryonic stem cells , thought to be a key factor in maintaining pluripotency.

NGFR: Nerve growth factor-receptor.

Oct4: Octamer-binding transcription factor 4.

OD: Optical density.

OSE: Ovarian surface epithelial cells.

PBS: Phosphate buffered saline.

PCR: Polymerase chain reaction.

PDGF: Platelet-derived growth factor.

PDGFR: Platelet-derived growth factor-receptor.

PDX-1: Pancreatic specific transcription factor-1.

POF: Premature ovarian failure.

PM: Phosphoramide mustard.

PSCs: Putative stem cells.

rpm: Round per minute

Sca-1: Stem cell antigen -1.

SCAP: Stem cells from apical papilla.

SCF: Stem cell factor.

SHED: Stem cells from human exfoliated deciduous teeth.

SOD: Superoxide Dismutase.

Sp-1: First sampling stage.

Sp-2: second sampling stage.

Sox2: (Sex determining region Y)-box 2.

SP: Side population.